

Transcriptional dysregulation in Huntington's disease

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Although the gene responsible for Huntington's disease was discovered in 1993, the pathogenic mechanisms by which mutant huntingtin causes neuronal dysfunction and death remain unclear. However, increasing evidence suggests that mutant huntingtin disrupts the normal transcriptional program of susceptible neurons. Thus, transcriptional dysregulation might be an important pathogenic mechanism in Huntington's disease.

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HUNTINGTON'S DISEASE (HD) is an autosomal dominantly inherited progressive neurodegenerative disease with a distinct topography of neuropathological damage^{1,2}. Although peripheral abnormalities have been described, HD is primarily a disease of the CNS. Within the CNS, brain structures are affected to varying degrees, with the medium spiny neurons of the caudate-putamen (striatum) being the most susceptible to degeneration³. Several hypotheses have been offered to account for the predilection of the striatum for damage in HD, but the striking regional selectivity remains inadequately explained. It was hoped that identification of the gene responsible for HD would reveal why specific areas of the CNS become preferentially damaged in individuals with HD. However, the *HD* gene, which encodes the protein huntingtin is expressed ubiquitously, not only throughout the brain but also in peripheral tissues^{4–8}. Thus, the distribution of huntingtin is itself insufficient to explain the neuropathological pattern of damage that occurs in HD. Instead, it is more likely that the damage is caused by the effects produced by the mutant form of this protein in individual cell types.

Huntingtin is a polyglutamine-containing protein

The mutation in the *HD* gene is an expansion of a CAG repeat, a trinucleotide motif that encodes a polyglutamine stretch within the mature protein⁴. Although huntingtin has no clear homology to any known protein, several other polyglutamine-containing proteins have been identified. Abnormal expansion of such proteins occurs in other neurodegenerative diseases^{9–12}. The CAG-repeat diseases share striking common features, including adult onset, progressive neurodegeneration, generational anticipation and a remarkably common threshold-expansion length, suggesting that these diseases share a common pathogenetic mechanism^{9,12–15}. Apart from the polyglutamine motif, the CAG-repeat disease proteins share no homology. Ataxin1, the abnormal protein in spinocerebellar ataxia type-1 (SCA1); (Refs 16,17), atrophin1, the abnormal protein in dentatorubropallidolusian atrophy¹⁸, and ataxin3, the abnormal protein in Machado-Joseph disease (SCA3) (Refs 19,20), are mainly found in the cell nucleus, although their 'normal' functions remain unknown. In one of the CAG-repeat diseases, spinobulbar muscular atrophy

(SBMA, Kennedy's disease), the function of the disease protein, the androgen receptor, is known and the mutant protein carries an abnormally expanded polyglutamine segment²¹. This receptor normally functions as a nuclear transcription factor, and the polyglutamine-expanded androgen receptor activates androgen-responsive reporter genes subnormally^{22–25}.

Many other proteins containing polyglutamine-rich regions are themselves transcription factors^{26–28}. These polyglutamine-rich regions can act as activation domains for transcription factors *in vitro*²⁶. Thus, when the mutant form of the *HD* gene expresses an abnormally long polyglutamine moiety, it might resemble a transcription factor. Other proteins that contain significant polyglutamine stretches include the transcriptionally active molecules N-Oct3 (a nervous-system-specific POU domain transcription factor); hSNF2a (a transcriptional co-activator for glucocorticoid, estrogen, and retinoic acid receptors); TATA-binding protein (TBP), (a transcriptional activator); amplified in breast cancer (AIB1), (a steroid receptor co-activator); TRAM1 (a thyroid hormone receptor activator); RAC3 (a transcriptional co-activator with intrinsic histone acetyl-transferase activity); nuclear receptor co-activator (ACTR), (a histone acetyltransferase and transcriptional cofactor); ATBFL (a zinc-finger homeodomain protein) and CREB-binding protein (CBP), (a transcriptional co-activator with histone acetylase function) (Refs 27,28). Furthermore, one of the most specific antibodies recognizing the expanded form of huntingtin is the 1C2 antibody, originally raised against TBP (Ref. 29). Thus, polyglutamines might have a role either in protein–DNA interactions or in the protein–protein interactions that occur when transcription factors form transcriptionally active complexes. Interestingly, a recent report showed that expansion of the CAG trinucleotide repeat within TBP was the cause of a neurological disease³⁰.

The recurrence of polyglutamine tracts in transcription factors suggests several theoretical transcriptional possibilities with regard to the function of mutant huntingtin. (1) The increased polyglutamine stretch might confer a 'gain of function' property resulting in direct binding of the mutant protein to DNA. Thus, when abnormally bound to DNA, mutant huntingtin might disrupt the normal pattern of transcription. (2) Huntingtin might bind aberrantly to specific transcription factor proteins, forming an

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inactive transcriptional complex that functions essentially as a repressor. (3) Huntingtin might form complexes with co-repressor proteins and therefore aberrantly de-repress transcription of normally silent genes. (4) Huntingtin might sequester transcription factors through aberrant interactions, depleting the levels of required factors within the cell. Although huntingtin has not been shown to alter transcriptional regulation in HD, in *in vitro* assays the polyglutamine-expanded form of huntingtin has been shown to be able to directly repress transcription of reporter gene constructs (Ref. 31; L. Jones, unpublished observations).

Abnormal nuclear localization of mutant huntingtin

Normal huntingtin is localized in the cytoplasm³²⁻³⁴ but mutant huntingtin in addition to being found in the cytoplasm is also found localized in the nucleus. In transgenic mice expressing exon 1 of the mutated human *HD* gene, nuclear translocation of the mutant protein is associated with increased huntingtin immunoreactivity, first diffusely within the nucleus and then around the nuclear pores. Invagination of the nuclear membrane is also observed³⁵. The processes governing nuclear translocation are unclear, although higher numbers of polyglutamine residues produce more nuclear localization in a variety of experimental systems³⁶⁻⁴². Insertion of a polyglutamine coding sequence into hypoxanthine phosphoribosyltransferase (*Hprt*) gene, which encodes a cytoplasmic protein without a polyglutamine moiety, results in translocation to the nucleus⁴³. Although polyglutamine repeat length seems to be one feature governing nuclear entry of huntingtin, another is the length of the whole molecule. Shorter fragments of huntingtin are translocated to the nucleus more efficiently, with longer fragments tending to form aggregates in the cytoplasm^{36,41}. In transgenic mouse models, lines that synthesize truncated forms of huntingtin^{44,45} demonstrate more nuclear inclusion formation and more abnormal phenotypic behavior than transgenic lines that synthesize full-length huntingtin^{42,46,47}. Similar results have been obtained in cellular models and in *Drosophila* models^{36,48}. It is not clear if huntingtin possesses active nuclear localization signals (NLS). However, in cellular experimental models, addition of nuclear export signals greatly reduces nuclear accumulation of huntingtin. Nuclear localization of huntingtin corresponded to increased cellular toxicity, although the mechanism by which this causes cellular damage is not yet clear⁴⁹⁻⁵¹.

One popular hypothesis is that full-length huntingtin undergoes a proteolytic cleavage event before translocating to the nucleus. In cell-culture models, transcripts of huntingtin in which caspase cleavage sites have been mutated demonstrate less nuclear localization and show increased survival (C. Wellington and M. Hayden, unpublished observations). Truncated forms of huntingtin are also more likely to form insoluble aggregates *in vitro*⁵². When transgenic mice expressing exon 1 of the mutated human *HD* gene are crossed with mice that possessing a dominant negative form of caspase-1, neuronal intranuclear inclusion formation is delayed, with a corresponding delay of symptom onset and prolongation of survival, suggesting

association between nuclear localization and cellular toxicity⁵³.

Abnormal protein interactions of mutant huntingtin

A consistent finding is that mutant huntingtin has altered protein-protein interactions compared with wild type huntingtin⁵⁴⁻⁶³. Both increased and decreased binding interactions of mutant huntingtin have been described. Yeast two-hybrid studies have uncovered numerous huntingtin-interacting proteins, many of which have altered interactions depending on the length of the polyglutamine moiety^{59,64}. In addition, many of these interactors are novel proteins with an as-yet undefined function⁵⁹. A possible mechanism for these interactions is the formation of polar-zipper structures, which have been postulated for polyglutamine-containing proteins⁶⁵. Another mechanism that has been suggested is transglutamination, with polyglutamine serving as a substrate for the enzyme transglutaminase⁶⁶⁻⁶⁸. A third mechanism is SH3 domain-dependent association of huntingtin, based on the presence of multiple proline-rich motifs, with SH3 domain-containing proteins, for example, Grb 2, RasGAP and epidermal growth-factor receptor^{57,61}.

Although a variety of enzymatic processes have been invoked for the formation of aggregates, including activation of caspases (the apoptotic proteases), transglutaminase and the proteasome-ubiquitin pathway, it is clear that abnormal huntingtin can self-polymerize *in vitro*, in the absence of enzymes⁵². Abnormal protein-protein interactions probably serve as the basis for the formation of abnormal huntingtin aggregates that have been observed in brains of individuals with HD (Refs 69-71), in transgenic mouse models^{48,50,72,73}, in *Drosophila* models^{51,74} and in cellular models of HD (Refs 37,38,40,44,45). Although the pathogenic role of abnormal inclusions has been debated^{17,44,45}, they probably represent a reliable pathogenic marker for an abnormal process. Abnormal aggregates of polyglutamine proteins are a common feature of the CAG trinucleotide repeats disorders, further suggesting that these diseases share a common pathogenic mechanism^{14,15}.

In addition, mutant huntingtin is able to recruit normal huntingtin into insoluble aggregates both *in vitro* and *in vivo*^{42,75}. In *Drosophila*, polyglutamine expansions alone, independent of protein context, mediate extreme cytotoxicity, neuronal degeneration and early adult death⁵¹. For example, when expanded polyglutamines are exogenously inserted into *Hprt*, neuronal intranuclear inclusions develop and mice develop an HD-like neurological phenotype⁴³. This intrinsic toxicity is dependent on cell type and polyglutamine length, and varies with the protein in which the polyglutamines are inserted, with certain host proteins able to neutralize the toxic effects.

Other proteins have been co-localized in polyglutamine inclusions, including ubiquitin, heat shock protein (HSP) 70, HSP90, HDJ-2/HSDJ, DNAJ, caspase-8 and several functional subunits of the proteasome^{35,72,76-78}. Although proteasomal dysfunction has yet to be convincingly demonstrated in HD, sequestration of proteasomal subunits could conceivably alter transcriptional regulation because several transcription factors, including activation of NF- κ B (Refs 79,80) and p53 (Refs 81-83), are dependent upon

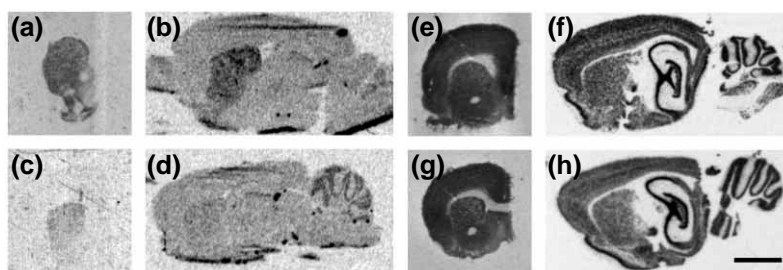
proteasomal processing^{84–86}. In chemical lesion models of HD, activation of the transcription factor NF- κ B contributes to excitotoxin-induced apoptosis^{87,88}. In addition, intracellular signaling pathways are themselves dependent upon the ubiquitin–proteasomal system for proper functioning⁸⁹.

The combination of aberrant nuclear localization and altered protein–protein interactions of mutant huntingtin suggests that mutant huntingtin might interact with nuclear proteins. Mutant huntingtin can recruit transcription factors into aggregates. For example, recruitment of TBP into aggregates has been shown *in vitro* as well as in human HD post-mortem brains⁷⁵. Mutant huntingtin has also been shown to recruit CBP into aggregates *in vitro*⁹⁰ and CBP is recruited into aggregates with the androgen receptor in cell culture, in a mouse model of SBMA, and in SBMA samples from humans⁹¹. Similarly, atrophin, the abnormal protein in the polyglutamine disease, dentatorubropallidoluysian atrophy (DRPLA), can recruit Eto. Eto is a transcriptionally active molecule, which is part of the histone deacetylase complex involved in transcriptional repression⁹². In addition, an interaction between the N-terminal pathogenic region of huntingtin and p53 has been shown *in vitro* by co-immunoprecipitation and observation of co-aggregation within inclusions generated in cell culture³¹.

Interactions of mutant huntingtin with transcriptional repressors have also been shown. A yeast two-hybrid system was used to identify an interaction of huntingtin with the transcription factor, nuclear co-repressor (N-CoR); (Refs 63,93). N-CoR is part of a complex that represses transcriptional in combination with several specific DNA-binding transcriptional repressors, including the thyroid hormone receptor, retinoic acid receptors, Mad:Max dimers and some of the ‘orphan’ nuclear receptors. N-CoR and mSin3 proteins link specific DNA-binding proteins to proteins with histone deacetylase activity. Immunohistochemical staining patterns for N-CoR and mSin3A are altered in the brains of patients with HD (Ref.63). In control human brains, neurons demonstrate N-CoR and mSin3A immunoreactivity in both the nucleus and the cytoplasm. By contrast, in brains of HD patients, N-CoR and mSin3A immunoreactivity is excluded from the nucleus, suggesting a redistribution of these transcription factors. A subset of intranuclear inclusions in HD brain samples demonstrate immunoreactivity for mSin3A (Ref. 63). These interactions support the possibility that huntingtin and other CAG-repeat disease proteins might affect cellular functioning via alteration of the normal expression pattern of genes, through abnormal association, intracellular redistribution and/or sequestration of crucial transcription factors.

Neurotransmitter receptor levels are altered in HD

Neurotransmitter alterations have been described in early-stage human HD autopsy material; many of these changes have been confirmed in transgenic mouse models of HD (Refs 94–97). In transgenic HD mice, neurotransmitter receptors are affected selectively; downregulation of specific receptors argues against a generalized problem with receptor production. This receptor downregulation occurs only in



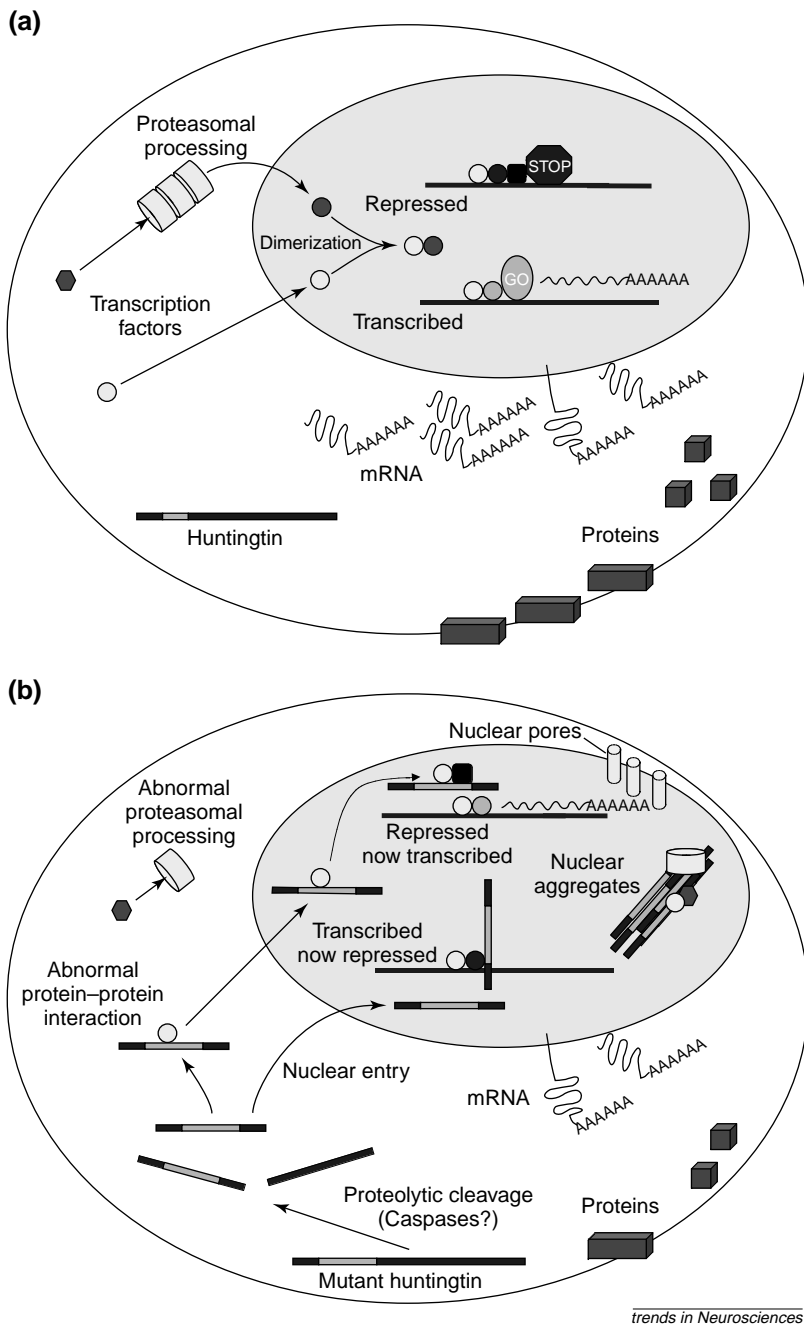
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Fig. 1. Selective alteration of neurotransmitter receptor levels in transgenic mice expressing exon 1 of mutated huntingtin. Autoradiographic images of adenosine A2a-receptor binding (using [³H]CGS-21680 as a ligand) in coronal sections of 12-week-old control mouse brain (a), and R6/2 transgenic mouse brain (c) showing decreased adenosine A2a receptor binding in R6/2 mice. Autoradiographic images of in situ hybridization of adenosine A2a receptor mRNA in sagittal sections of control mouse brain (b), and R6/2 transgenic mouse brain (d), demonstrating decreased adenosine A2a receptors mRNA levels in R6/2 mice. By contrast, receptor binding autoradiography for NMDA receptors shows that there is no difference between coronal sections of control brain (e), and R6/2 transgenic mouse brain (g). Similarly, in situ hybridization for the NR1 subunit mRNA of the NMDA receptor shows that there is no difference between sagittal sections of control mouse brain (f) and R6/2 transgenic mouse brain (h). Scale bar, 2 mm. Neurotransmitter receptor alterations observed in the brains of transgenic mice parallel those that have been described in human Huntington's disease.

transgenic lines with abnormal CAG-repeat numbers⁹⁷. Decreases in receptor binding are corroborated by decreases in receptor mRNA levels, indicating that affected receptors decrease in number, rather than changing their binding characteristics (Fig. 1). Because receptor downregulation occurs before the onset of symptoms or observable cell loss, receptor decreases are not merely epiphenomena of disease progression, supporting the notion that neuronal dysfunction is just as relevant for the etiology of symptoms as neuronal death. Especially prominent are early decreases in the levels of dopamine D₁ and D₂ receptors, both of which regulate gene expression, suggesting that certain alterations in mRNA levels observed in HD are secondary to the loss of neurotransmitter receptors^{98,99}. Although decreased mRNA levels suggest that receptor alteration occurs at the level of gene transcription, these observations cannot exclude the possibility that these changes occur at the level of mRNA degradation. However, the observation that many mRNA transcripts are unchanged argues against a general defect with mRNA processing.

Receptor changes first appear before the onset of observable symptoms in transgenic HD mice but the magnitude and number of downregulated receptors increases over time, implying a progressive mechanism. One possibility is that progressive receptor downregulation is a functional correlate of the increasing amount of nuclear-localized mutant huntingtin or progressive inclusion formation. In HD mice crossed with caspase-1 dominant negative mice, delayed symptom onset and prolonged survival correspond not only to delayed nuclear inclusion formation, but also to delayed loss of neurotransmitter receptor binding and a delayed decrease of the corresponding receptor mRNAs (Ref. 53).

One of the surprising conclusions about these observations, is that a single mutant gene can affect the expression of multiple neurotransmitter receptors. Mechanistically, this observation increases the likelihood that mutant huntingtin acts by affecting a crucial central mechanism. Mutant huntingtin probably



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Fig. 2. Potential mechanisms of transcriptional dysregulation in Huntington's disease. (a) In a normal neuron, in which huntingtin is a cytoplasmic protein, nuclear genes are appropriately expressed or repressed, mRNAs are transcribed from which the normal complement of membrane and cytoplasmic proteins are produced. (b) In a neuron of an individual with Huntington's disease, mutant huntingtin contains an expanded polyglutamine which might be cleaved by effector caspases, thereby generating toxic fragments. Truncated fragments of mutant huntingtin can then aberrantly localize to the nucleus and participate in abnormal protein-protein interactions both in the cytoplasm and in the nucleus. Aberrant interactions with transcription factors might compromise basal transcription. Nuclear aggregates of mutant huntingtin fragments recruit proteasomal subunits and transcription factors, thus depleting the cell of crucial factors. This nuclear disruption results in the appearance of nuclear pores and a decrease in the generation of specific receptor mRNA levels and a correlative decrease in the receptor proteins.

exerts its deleterious actions in the nucleus by disrupting the normal expression of specific genes. Thus, the regional specificity of the neuropathological damage seen in HD might not be a function of where huntingtin is found, but rather, of the total set of genes whose transcription is altered by mutant huntingtin¹⁰⁰. For example, the gene defect in HD might not directly kill neurons but might contribute to striatal toxicity by

promoting release of glutamate from corticostriatal terminals, by altering signaling or by altering transcription of proteins involved in excitotoxicity^{96,101}.

Mutant huntingtin disrupts transcription of specific genes

There is no *a priori* reason to suggest that mutant huntingtin-induced transcriptional dysregulation is limited to neurotransmitter receptor genes. Several groups have now found evidence that huntingtin affects the expression of several genes. For example, in PC12 cells transfected with exon 1 of mutated huntingtin, differential display RT-PCR was used to reveal the presence of numerous altered transcripts¹⁰². Examples were found of mRNAs that had decreased as well as those that had increased, although a higher number showed a decrease in mRNA levels. Production of polyglutamine-expanded huntingtin has also been shown to activate the stress-signaling kinase (SEK1)-JUN N-terminal kinase (JNK) pathway and induce apoptosis in a hippocampal neuronal cell line¹⁰³. JNK is known to activate Jun and AP-1 transcription factors in neuronal apoptosis.

Gene expression arrays on DNA microchips have recently been used to examine the scope of transcriptional changes in transgenic HD mice¹⁰⁴. Using the murine Mu6500 chip (Affymetrix, Santa Clara, CA, USA) mRNA transcripts from striata of 6- and 12-week-old R6/2 mice were compared with striatal transcripts from age-matched controls. The results showed that the genes whose expression levels were altered were limited to several key molecular systems. The affected systems included neurotransmitter receptors, intracellular signaling mechanisms, retinoic acid receptor machinery and calcium homeostasis systems. Overall, only 1.2% of the genes examined had altered levels of expression. Gene expression changes occurred at an early pre-symptomatic time-point (6-weeks of age), and similar pathways were affected at a later symptomatic time point (12-weeks of age), indicating that the altered expression of this set of genes remained stable. A progressive increase in the number of genes affected was reminiscent of the increase in the magnitude and number of downregulated neurotransmitter receptor genes. Interestingly, there was no change in cytoskeletal proteins, enzymes of intermediary metabolism, caspases or mitochondrial proteins. The pattern of affected genes shows that a single mutant protein affects the transcription of many genes. In addition, the set of genes whose expression levels are altered gives us an insight into why HD is a neural disease: the affected genes are especially relevant to neuronal functioning (Fig. 2). A recent report of altered gene expression in transgenic SCA1 mouse brain supports this view¹⁰⁵. These authors found specific decreases in the expression of multiple signal transduction and calcium homeostasis genes before the onset of symptoms.

Concluding remarks

In spite of the discovery of the huntingtin gene in 1993, the mechanisms by which mutant huntingtin exerts its toxic effects remain unknown. The anatomical distribution of the expression of huntingtin is insufficient to account for the pattern of neuropathological damage observed in HD. Clinical neurological symptoms might have as their basis, altered

neurotransmitter receptor levels and disrupted synaptic transmission. Transcriptional dysregulation is emerging as a probable pathogenic mechanism, not only in HD, but also in other polyglutamine diseases. Mutant huntingtin, by virtue of the expanded polyglutamine moiety, has structural similarities to known transcription factors. It also demonstrates aberrant nuclear translocation, occasionally resulting in aggregate formation. Nuclear localization of mutant huntingtin appears to be required for cellular toxicity. Human HD and transgenic mouse models of HD demonstrate downregulation of certain neurotransmitter receptor genes that occur at the level of mRNA expression. Therefore it is probable that mutant huntingtin exerts its toxic effects by affecting the expression of a set of genes that are important in neural and striatal functioning. The altered expression of this set of genes probably leads to the cellular dysfunction and eventual neuronal death which occurs in HD. Deciphering the spectrum of mutant huntingtin-induced transcriptional dysregulation promises not only to inform our understanding of the striking regional neuropathology of HD, but also to define novel targets for therapeutic intervention. Thus each identified gene whose expression is altered in HD provides a potential opportunity for developing a treatment for this dreadful disease.

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