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The Early Cellular Pathology of Huntington's Disease

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Abstract

Huntington's disease (HD) is an inherited neurodegenerative disorder that affects about one in 10,000 individuals in North America. The genetic defect responsible for the disease is an expansion of a CAG repeat that encodes a polyglutamine tract in the expressed protein, huntingtin. The disease is characterized by involuntary movements, cognitive impairment, and emotional disturbance. Despite the widespread expression of huntingtin, the brains of HD patients show selective neuronal loss in the striatum and the deep layers of the cerebral cortex. Recent studies have shown that polyglutamine expansion causes huntingtin to aggregate, to accumulate in the nucleus, and to interact abnormally with other proteins. Several cellular and animal models for HD have revealed that intranuclear accumulation of mutant huntingtin and the formation of neuropil aggregates precede neurological symptoms and neurodegeneration. Intranuclear huntingtin may affect nuclear function and the expression of genes important for neuronal function, whereas neuropil aggregates may interfere with neuritic transport and function. These early pathological events, which occur in the absence of neurodegeneration, may contribute to the neurological symptoms of HD and ultimately lead to neuronal cell death.

Index Entries: Huntington's disease; huntingtin; polyglutamine; aggregates; gene expression; neurodegeneration; pathogenesis; neuropil.

Introduction

Expansion of a CAG repeat has been found to cause eight inherited neurodegenerative diseases: Huntington's disease (HD) *(1)*; dentatorubral and pallidoluysian atrophy (DRPLA) *(2)*; spinal and bulbar muscular atrophy (SBMA) *(3)*; and several forms of spinocerebellar ataxia (SCA1) *(4)*, SCA2 *(5–7)*, SCA3 *(7a)*, SCA6 *(7b)*, and SCA7 *(7c)*. The CAG repeat contains 10 to 35 units in normal individuals and more than 37 units in HD patients *(1)*. Most HD cases are adult patients with repeat lengths between 40 and 55 units; juvenile HD patients have expanded repeat lengths of over 60 units *(8)*. A good inverse correlation between CAG repeat length and age at onset of neurological symptoms is observed among

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patients with repeats longer than 60 units *(9–11)*. The neurological symptoms of HD are involuntary movements, cognitive impairment, and emotional disturbance; symptoms progress until death *(12,13)*.

Exon 1 of the HD gene contains a CAG repeat that is translated into a polyglutamine (polyQ) tract in the N-terminal region of huntingtin, a 350 kDa protein of unknown function *(1)*. Normal huntingtin is a cytoplasmic protein and is expressed ubiquitously, but the neuropathology of HD is consistent with the selective neuronal loss observed in the striatum and cerebral cortex of patients' brains *(14)*. Recent studies of huntingtin and other polyQ proteins have greatly advanced our knowledge of huntingtin's expanded polyQ and its related neuropathology. First, transgenic mice expressing the HD exon1 protein (R6/2) with more than 115 glutamines develop neurological symptoms and neuronal intranuclear inclusions consisting of huntingtin aggregates *(15)*. Insertion of an expanded polyQ can cause a nonpathogenic protein, hypoxanthine phosphoribosyltransferase (HPRT), to form intranuclear aggregates; mice carrying this mutant protein develop a progressive neurological disorder *(16)*. Other types of HD transgenic mice expressing expanded polyQ also show that behavioral and movement disorders can occur in the absence of neurodegeneration *(17–19)*.

Neurodegeneration occurs at the late stage of HD and, thus, may be the result of cumulative effects of multiple pathologic events. Although it is evident that polyQ expansion confers this pathogenic property, the pathways leading to neurodegeneration remain to be elucidated. Neuronal intranuclear inclusions (NII) have been thought to be the cause of the disease, because their formation is correlated with the length of the repeat *(20–22)* and precedes the onset of neurological symptoms in HD transgenic mice *(15)*. Moreover, similar aggregates are found in the brains of HD patients *(23,24)* and patients with other polyQ diseases such as DRPLA *(24,25)*, SBMA *(26,27)*, SCA1 *(28,29)*, SCA2 *(30)*, SCA3 *(31,32)*, SCA6 *(33)*, and SCA7 *(34)*. The presence of huntingtin aggregates is also correlated with cell death in various types of transiently transfected cells *(20,22,35)*. However, the hypothesis that aggregates cause cell death has recently been challenged by studies showing that the nuclear localization of polyQ proteins, not the formation of aggregates, induces neuronal pathology *(36–38)*. Further, although huntingtin aggregates are present in the affected areas of HD brains, huntingtin aggregates in the striatum are not found in spatial patterns coinciding with neuronal loss in HD *(39)*. Other cellular and animal models have also suggested that cellular function is impaired by polyQ proteins themselves, before the detection of neurodegeneration, cell death, or protein aggregation *(40–42)*. Because understanding the early pathology of HD will help elucidate the specific neuropathology of HD and help develop an effective therapeutic strategy, this review focuses on the pathological events that precede obvious neurodegeneration, or occur in the absence of obvious aggregates. The possible roles of intranuclear huntingtin and neuropil aggregates in HD pathogenesis will also be discussed.

HD Transgenic Mice Develop a Neurological Phenotype in the Absence of Neuronal Degeneration

The first HD animal model, the R6/2 mouse, was established by introducing a transgene consisting of the human HD promoter region and exon1. The transgenic protein contains 67 amino acids of N-terminal huntingtin with 141–157 glutamines *(43)*. Beginning as early as 5 wk of age, these mice develop a progressive neurological phenotype that includes behavioral and motor symptoms including a resting tremor, abrupt shuddering movements, stereotypic grooming movements, and, in some cases, epileptic seizures *(15,44)*. In addition, brain weight begins to decrease at 4 wk of age, and body weight begins to decrease at 8 wk of

Table 1
Transgenic-Mouse Models for Huntington's Disease

Construct	Promoter	Number of CAGs	Symptoms[a]	Onset of symptoms (wk after birth)	Neuro-degeneration[b]	Refs.
Exon1 (67 aa)	Human HD	115–156	Yes	5	None	*(15)* *(44)*
N-terminal (171 aa)	Mouse prion	82	Yes	12–20	None	*(19)*
Full-length	CMV	48 and 89	Yes	8	Yes (24 wk)	*(80)*
YAC full-length	Human HD	72	Yes	12	Yes (48 wk)	*(18)*
Knock-in full-length	Mouse HD	72–80	Yes	12	None	*(47)*
Knock-in full-length	Mouse HD	50	None	None	None	*(46)*

[a] Symptoms are behavioral or motor deficits examined.

[b] Number in parenthesis indicates the earliest age of animals examined.

age. Most importantly, the transgene protein forms NII that consist of aggregated huntingtin *(15)*. The R6/2 mice usually die at 12 wk without showing neurodegeneration. However, selective neurodegeneration can be found in those mice that have survived beyond 14 wk *(45)*. In mice expressing a longer N-terminal fragment of huntingtin (171 amino acids) with 82 glutamines, similar behavioral and motor abnormalities are also observed, but slightly later, at 12–20 wk of age. These abnormalities include loss of coordination, tremors, and hypokinesis. However, no neuronal loss was detected in the symptomatic mice *(19)*.

The rapid development of a neurological phenotype in mice expressing N-terminal fragments of huntingtin suggests that N-terminal huntingtin with expanded polyQ is toxic to neurons. Interestingly, transgenic mice expressing full-length mutant huntingtin develop a less severe phenotype. For instance, yeast artificial chromosome (YAC) transgenic mice expressing mutant huntingtin with 72 glutamines (YAC 72) show obvious circling and choreoathetoid movement at the age of 9 mos, though the earliest behavioral symptom can be detected at 3 mo *(18)*. Hence, it appears that the N-terminal fragment of expanded polyQ huntingtin is more toxic than a full-length protein with a similar expansion.

The expression level of mutant huntingtin could also affect the development of a neurological phenotype. Overexpression of full-length huntingtin with 89 glutamines, driven by a heterologous cytomegalovirus promoter, resulted in mice that manifested progressive behavioral and motor dysfunction at 8 wk of age. These early behavioral abnormalities include trunk curling and clasping of both fore- and hindlimbs. Although these mice show neuronal loss in the striatum, cerebral cortex, thalamus, and hippocampus, clear neurodegeneration was evident only after the onset of feet-clasping and hyperkinesia *(17)*. Two HD repeat knock-in mice were created by introducing an expanded CAG repeat (50 or 80 CAGs) into the endogenous mouse HD locus *(46,47)*. Although these mice do not show a dramatic phenotype, the ones expressing 80 CAG repeats display abnormal social behavioral after 12 wk. No neurodegeneration is evident in these mice, even at the age of 17 mo *(47)*. Taken together, these in vivo models indicate that mutant huntingtin can mediate early neurological symptoms prior to neurodegeneration (Table 1). Consistent with this notion, some HD patients also had years of chorea, even though their postmortem brains did not show obvious neurodegeneration *(14,48)*.

Early Pathological Events in the HD Brain

In R6/2 mice, NII form before the onset of symptoms, leading to the hypothesis that NII may play a causative role in HD *(15)*. Huntingtin with a polyQ expansion in the pathological range (51 glutamines) can even form amyloid-like aggregates in vitro *(49)*. Subsequently, DiFiglia et al. identified a structure similar to NII in HD patient brain cells *(23)*. Interestingly, extranuclear huntingtin aggregates, named dystrophic neurites, were also found in HD brains. Dystrophic neurites were thought to be huntingtin aggregates formed in degenerated neuronal processes. Interestingly, dystrophic neurites, but not NII, were found in the brain of a presymptomatic patient, suggesting that they may be more important than NII in the onset of neurological HD symptoms *(23)*.

To characterize further huntingtin aggregates in HD brain, Li et al. generated a polyclonal antibody (PAb) using a GST fusion protein antigen that contains the first 256 amino acids of huntingtin with a deletion of the polyQ and polyproline stretches. This antibody should react specifically with huntingtin, as the deletion of the repeats in the antigen will prevent cross immunoreactivity with other polyQ proteins. Interestingly, this antibody (EM48) preferentially binds to huntingtin containing expanded polyQ, providing a unique means to detect mutant huntingtin and huntingtin aggregates. EM48 immunostaining of HD brain tissues was performed in a collaborative work by Drs. Gutekunst, Hersch, Li, et al. at Emory University *(39)*. The study revealed more aggregates than did previous reports using other antibodies. Many small aggregates are localized in neuronal processes and were named neuropil aggregates. Unlike NII or intranuclear aggregates, small neuropil aggregates are not labeled by anti-ubiquitin antibody, suggesting that they have not been ubiquitinated. Electron microscopic examination verified that neuropil aggregates are within dendrites (Fig. 1). Identification of neuropil aggregates also suggests that the previously described dystrophic neurites are derived from small neuropil aggregates *(39)*. Extending DiFiglia's observation *(23)* that NII and dystrophic neurites are formed by N-terminal huntingtin, EM48 immunocytochemistry shows that neuropil aggregates are only labeled by EM48, but not by an antibody against the internal region of huntingtin (amino acids 549–679). In addition, EM48 immunoreactive neuropil aggregates were very frequent in a presymptomatic HD patient in which no NII were detected *(39)*.

Neuropil Aggregate Formation Is Well-Correlated with Disease Progression

To characterize further the relationship between neuropil aggregates and disease progression, the density of neuropil aggregates was measured in HD patient postmortem brains that had various extents of neurodegeneration. Based on the degree of neuronal loss in the striatum, HD cases are classified as grades 0–4 *(14)*. Grades 0–2 represent early HD and grades 3–4 are late stage of HD. This grading system helps reveal some pathological events that are more likely associated with early HD. In grade 1 HD brain, a remarkably high density of neuropil aggregates was found in the cerebral cortex with almost no NII in this area. Neuropil aggregates in the cortex are more frequent in grade 1 than grade 4. NII or intranuclear aggregates, on the other hand, become more abundant in grades 3 and 4 HD brains. Despite their progressive formation in advanced HD brain, the distribution of NII in the striatum does not correspond to the selective neurodegeneration pattern in this region *(39)*. It appears that the presence of NII cannot explain the selectivity of striatal degeneration. On the other hand, neuropil aggregates seem to be more closely linked to neurological symptoms of HD than to neurodegeneration.

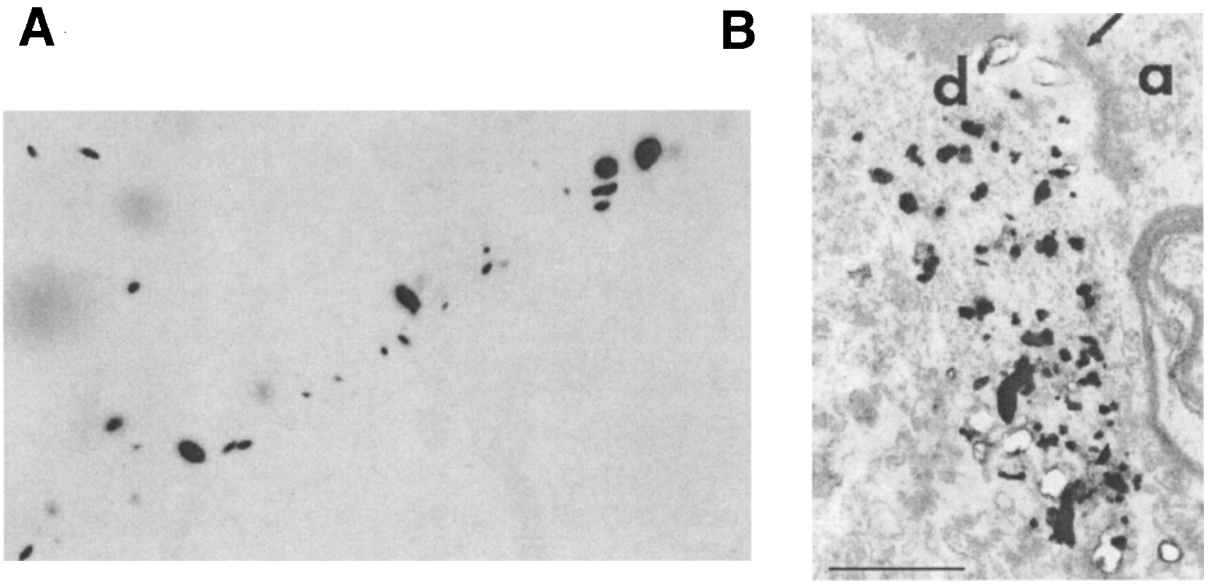

Fig. 1. EM48 immunoreactive neuropil aggregates in HD patient brain. **(A)** Light micrographs of HD cortex (grade 1) labeled with EM48. The antibody labels aggregates of different shapes and cellular localizations. In the neuropil, small spherical or fusiform aggregates are arrayed in rows along the length of the neuronal process. Scale bars, 10 µm. **(B)** Electron micrograph of EM48 immunogold-labeled aggregates in insular cortex from an adult HD brain (grade 1). Immunogold particles are associated with aggregates made of filamentous material within dendritic processes. d, dendrite; a, axon terminal. Scale bar, 500 nm. Adapted with permission from ref *(39)*.

Identification of EM48 immunoreactive neuropil aggregates in HD patient brains led to an examination of R6/2 mice using EM48 immunocytochemistry *(50)*. Several interesting observations were made. First, small neuropil aggregates are also present in these HD transgenic mice. Second, like those in HD patients' brains, most small neuropil aggregates in R6/2 mice are not labeled by anti-ubiquitin antibody. Third, small aggregates are localized in axons and axonal terminals, in addition to their localization in dendrites (Fig. 2). It is possible that the poorly preserved ultrastructure of the postmortem tissues blocked the detection of axonal aggregates in human brain *(39)*; the identification of axon aggregates in R6/2 mice implies that similar axon aggregates may be present in HD patients. Fourth, the progressive formation of neuropil aggregates is highly correlated with the development of neurological symptoms. For instance, in R6/2 mice, the density of neuropil aggregates increases by 13.6-fold between 4 and 12 wk after birth, whereas the density of NII increases by only 1.7-fold. Huntingtin aggregation apparently precedes the onset of the neurological symptoms and continues to progress in the absence of neurodegeneration (Table 2).

In mice expressing the N-terminal fragment (177 amino acids) of huntingtin with 82 glutamines, similar aggregates in neurites were also evident. These aggregates were identified by an anti-peptide antibody against the first 17 amino acids of human huntingtin *(19)*. NII were also present in these HD transgenic mice. However, both NII and neuropil aggregates were found in symptomatic mice without detectable neurodegeneration. Taken together, neurodegeneration appears to be a late stage of HD, which does not immediately follow huntingtin aggregation or early neurological symptoms.

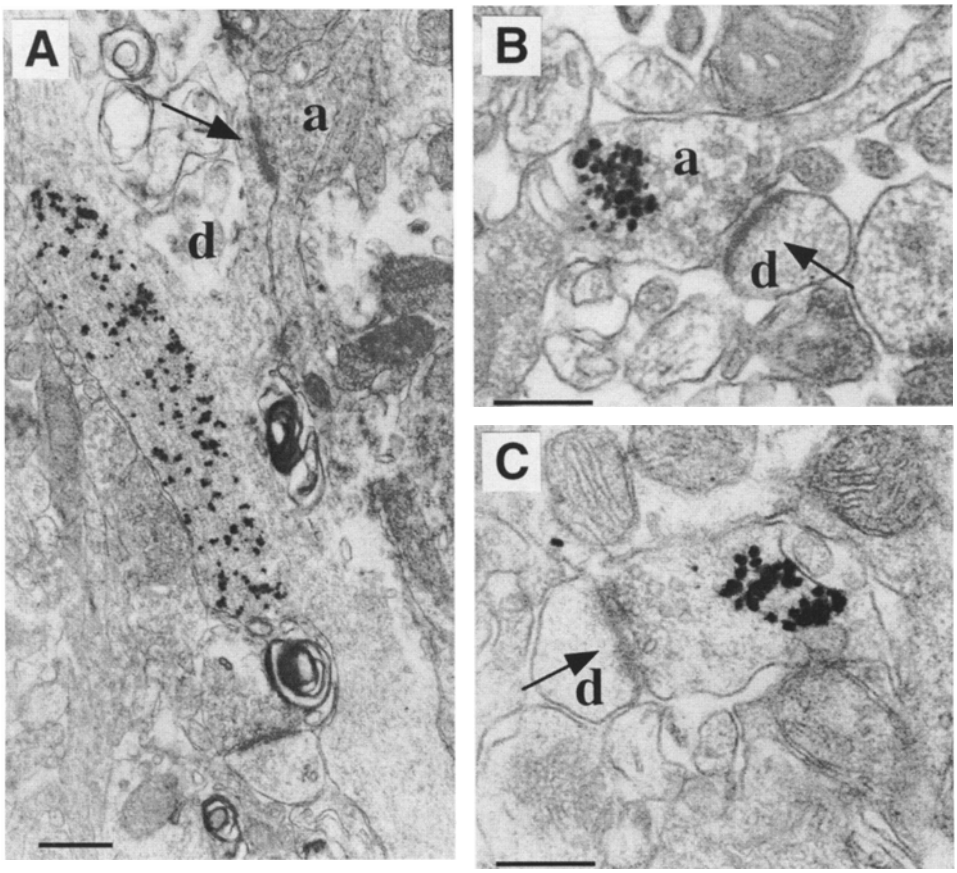

Fig. 2. Ultrastructural localization of mutant huntingtin in dendrites and axons in HD transgenic mice. **(A)** Electron micrographs of EM48 immunogold labeling in the cerebral cortex of an 8-wk R6/2 transgenic mouse. Immunogold particles are associated with an aggregate within a dendrite. Note that this dendrite contains a postsynaptic density (arrow) and contacts with an axon terminal in which synaptic vesicles are visible. The aggregate contains many fibrillated structures in which immunogold particles are highly enriched. Scale bars, 0.5 μm. **(B–C)** Immunogold particles are clustered in axon terminals in cortex **(B)** and striatum **(C).** Postsynaptic densities (arrows) and a dendrite are indicated. Some immunogold particles are clearly associated with synaptic vesicles in the terminal. d, dendrite, a, axon terminal. Scale bars, 0.25 μm. Adapted with permission from ref. *(50).*

Table 2
Increase in Neuropil or Nuclear Aggregate Formation
During Disease Progression

	Age in wk			
	4	6	8	12
Neuropil aggregates	1	3.3	7.3	13.6
Nuclear aggregates	1	1.4	1.6	1.7
Brain weight loss*	–	++	+++	+++
Body weight loss*	–	–	+	+++
Symptoms*	–	+	+	++

* Symptoms are motor deficits and disorders that were described by Davies et al. *(23)* and Carter et al. *(44).* The increase in aggregate formation is expressed as folds of the density of aggregates in 4-week R6/2 mice. Adapted with permission from ref. *(50).*

Intranuclear Localization of Huntingtin Is Sufficient to Mediate Cellular Pathology

Despite their abundance in HD brain, the role of NII remains controversial. However, it is evident that intranuclear accumulation of huntingtin is required for the formation of NII. In very young R6/2 mice (4 wk), a diffuse nuclear localization of transgenic protein is more apparent than NII. As the disease progresses, NII become predominant *(15,50)*. Similarly, in HD repeat knock-in *(51,52)* and YAC 72 transgenic mice *(18)* that express full-length mutant huntingtin, intranuclear localization of mutant huntingtin first occurs in the striatum, preceding the formation of obvious aggregates. The intranuclear localization, rather than aggregation, of mutant huntingtin has been shown to be sufficient to induce neuronal degeneration in cultured striatal neurons *(37)*. This study found that intranuclear aggregation of huntingtin was not associated with cell death. Instead, cell death occurs only when soluble transfected huntingtin is localized in the nucleus. Moreover, SCA 1 transgenic mice also show that the elimination of nuclear localization, but not the aggregation, of ataxin-1 with an expanded polyQ can prevent neuronal degeneration *(36)*.

A cellular model that expresses mutant huntingtin selectively in the nucleus will demonstrate the significance of nuclear localization. Rat pheochromocytoma (PC12) cells that stably express the huntingtin exon 1 protein with 150 glutamine repeats (150Q) were recently established *(42)*. As expected, the transgenic protein in this cell model is localized in the nucleus, as it is in R6/2 mice (Fig. 3A). In contrast to the transgenic huntingtin in R6/2 mice, the transfected huntingtin in cultured cells does not form aggregates in the nucleus, perhaps because cell division prevents its aggregation. Nevertheless, this cell model demonstrates the possibility of cellular pathology in the absence of obvious huntingtin aggregation. In control cells expressing the same HD exon1 protein with 20 glutamines (20Q), huntingtin is mainly diffuse in the cytoplasm. In 150Q cells, several cellular pathological phenomenon were found to associate with the intranuclear localization of mutant huntingtin. First, 150Q cells do not develop neurites in the presence of nerve

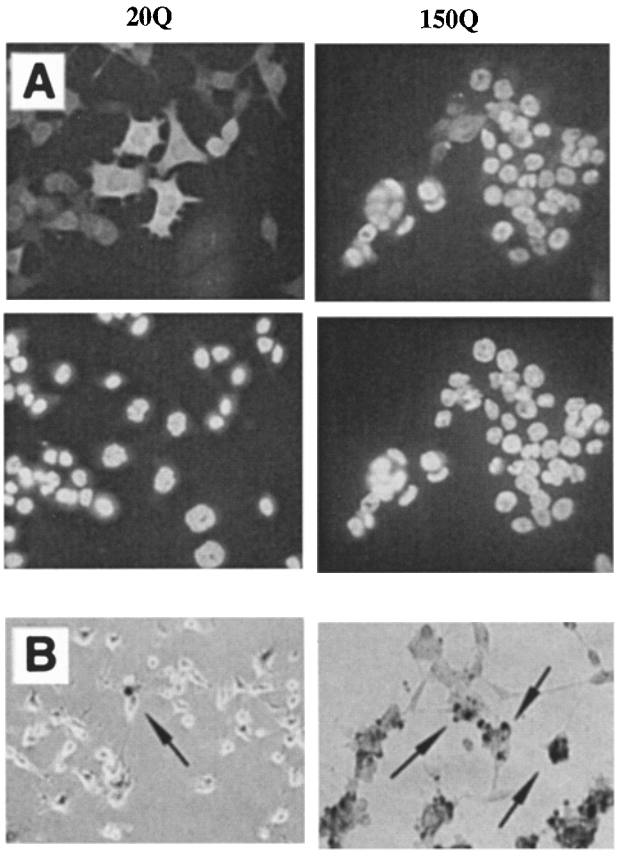

Fig. 3. Intranuclear transfected huntingtin and cell toxicity in stably transfected PC12 cells. **(A)** Immunofluorescent staining showing that EM48 intensely labels the transfected huntingtin in stably transfected PC12 cells that express 20Q or 150Q protein. The same cells in the lower panel were stained with Hoechst dye to reveal the nuclei. Note that 20Q is predominately distributed in the cytoplasm, whereas 150Q is concentrated in the nucleus. Scale bar, 10 μm. **(B)** Phase contrast images showing that staurosporine (100 nM) kills more 150Q cells than 20Q cells. Dead cells were stained with trypan blue stain (arrows). Adapted with permission from ref. *(42)*.

growth factors. However, these cells were able to grow neurites when treated with staurosporine, a drug that promotes neurite outgrowth by acting directly on intracellular signaling pathways *(53)*. The defective neurite outgrowth in 150Q cells may mirror some pathological events involved in neuropil degeneration in HD patient brains *(54–56)*. Second, 150Q cells are more susceptible to apoptotic stimulation by staurosporine and die faster than 20Q cells (Fig. 3B). The susceptibility of 150Q cells to apoptotic stimulation is consistent with the greater cell death observed in other huntingtin transfected cells *(20,22,37)*. Because 150Q cells can propagate for more than 50 passages without losing their phenotype, this HD cellular model provides further evidence showing that cellular pathology can occur in the absence of huntingtin aggregation and acute cell death.

Mechanisms of Early Pathology in HD

The intranuclear accumulation and aggregation of N-terminal mutant huntingtin reflect proteolytic cleavage of full-length huntingtin, which may be an initial step toward various pathological consequences. Although it is unknown how N-terminal huntingtin is translocated into the nucleus, the intranuclear environment has been found to accelerate the aggregation of polyQ proteins *(57)*. The aggregation of polyQ proteins is thought to be the result of polar zipper formation by glutamine residues *(58)* or the result of crosslinking by transglutaminase *(59)*. PolyQ aggregation is suppressed by cytoplasmic chaperonins and proteasomes *(60,61)*. Perhaps because these inhibitory proteins are lacking or reduced in the nucleus, mutant huntingtin preferentially forms intranuclear aggregates or NII. Interestingly, huntingtin aggregates are also abundant in neuronal processes. The unique architecture of neuronal processes and nerve terminals may allow the local concentration of huntingtin to reach readily the threshold at which aggregation occurs. Alternatively, misfolded huntingtin may have less access to chaperonins, proteasomes, or other inhibitory molecules in the processes than in the cell body.

In addition to aberrant nuclear accumulation and aggregation, abnormal interactions of mutant huntingtin with other proteins are also observed. For instance, polyQ expansion can enhance the interactions between huntingtin and several other proteins, including huntingtin-associated protein-1 (HAP1) *(62)*, glyceraldehyde phosphate dehydrogenase (GAPDH) *(63)*, calmodulin-associated proteins *(64)*, and an SH3 domain-containing protein, SH3GL3 *(65)*. These findings fit well into the hypothesis of the gain of function of polyQ expansion. Abnormal protein interactions with mutant huntingtin may constitute the molecular basis for the variety of cellular pathology that occurs in both the cytoplasm and the nucleus.

Intranuclear Huntingtin May Affect Gene Expression

One of very likely sources of HD pathology is an alteration of gene expression caused by intranuclear huntingtin. Evidence supporting this hypothesis comes from the finding that R6/2 mice have altered expression of a specific glutamate receptor, specifically, a downregulation of presynaptic mGluR2 receptor in their cerebral cortex *(66)*. In PC12 cells stably transfected with 150Q huntingtin, the decreased expression of several genes important for neuronal functions has been documented. These genes encode for NGF receptors (TrkA and p75); HAP1, a protein that may be involved in microtubule-based transport in neurons *(67)*; and metallothionein-II (MII), which protects cells from oxidative damage *(68,69)*. Interestingly, the expression of glutamate transporter (GLAST) is increased in 150Q cells. The decreased

expression of nerve growth factor receptors is consistent with the defective neurite differentiation observed in PC12 cells, whereas the decreased expression of MII and the increased expression of GLAST may be associated with cell death *(42)*.

The idea that intranuclear huntingtin alters gene expression is also suggested by the fact that many transcription factors are glutamine-rich proteins and that the polyglutamine domain can enhance the transcriptional activity of some of these factors *(70–72)*. Expanded polyQ-containing proteins have also been found to recruit other polyQ proteins, including TATA binding protein, a transcription factor that is involved in the vast majority of transcription *(57,73)*. Thus, it is reasonable to propose that intranuclear huntingtin with expanded polyQ interferes with gene expression by interacting with transcription factors. This possibility has been suggested by the findings that SCA1 protein binds to a nuclear protein LANP *(28,29)*. This idea also raises an interesting question of whether intranuclear huntingtin alters the expression of genes important for mitochondrial function and thus causes the impaired energy metabolism that has been observed in HD-affected neurons *(74)*.

Impaired Synaptic Function by Mutant Huntingtin or Aggregates

Because neuronal processes are a unique compartment of neurons, neuropil and axonal aggregates may be more relevant to HD neuropathology than intranuclear aggregates. Aggregates in neuronal processes or nerve terminals are likely to interfere mechanically with intracellular transport between the cell body and nerve terminals when they physically block the pathway or abnormally interact with other transporters. HAP1 may help to elucidate this process, because it binds to both huntingtin and dynactin p150, a dynein-associated protein that is involved in microtubule-based transport in neurons *(67,75)*. Aggregates in axon terminals could also directly affect the recycling of synaptic vesicles or synaptic transmission and therefore result in the impairment or dysfunction of neuronal communication and interaction. If so, such dysfunction could be responsible for the neurological symptoms that occur long before neurodegeneration. Consistent with this idea, YAC 72 transgenic mice and 80Q HD knock-in mice have shown impaired synaptic transmission *(18,76)*. In the hippocampal CA 1 neurons of YAC 72 mice, an increase in NMDA receptor function is detected at 6 mo, followed by a decrease in long-term potentiation (LTP) at 10 mo of age. The electrophysiological abnormalities precede any obvious symptoms, huntingtin aggregation, or neurodegeneration *(18.)* In 80Q HD repeat knock-in mice at 8–14 mo of age, LTP induction is also impaired in hippocampal slices *(76)*. Both studies suggest that mutant huntingtin acts in the presynaptic terminal to influence glutamate neurotransmitter release. In R6/2 mice, the majority (90–100%) of axonal aggregates are found in asymmetric synapses, which are likely to be excitatory and mainly contain glutamate neurotransmitters in their presynaptic vesicles *(50)*. This finding provides a morphological basis for the abovementioned suggestion, as well as the hypothesis of excitotoxicity in HD, which proposes that HD is associated with glutamate neurotoxicity *(77)*.

A puzzling issue about HD is how the disease leads to selective neurodegeneration in the striatum and deeper layers of the cerebral cortex. Selective neuronal loss may be contributed by tissue-specific factors involved in huntingtin processing, nuclear translocation, or protein aggregation. In YAC 72 transgenic mice and in HD repeat knock-in mice *(51,52)*, mutant huntingtin is first translocated into the nucleus of medium spiny neurons, a major type of striatal neurons that are vulnerable in HD. This finding suggests that striatal processing of mutant huntingtin may contribute to the early nuclear localization of huntingtin and the specific pathology in the striatum. However, in human HD brain and

R6/2 mice, more aggregates are observed in the cerebral cortex than in the striatum (*39,50*). Cortical neurons may preserve aggregates better than striatal neurons so they show more aggregates in postmortem brain and in R6/2 mice in which the HD exon1 protein forms aggregates without proteolytic processing. Nevertheless, the enrichment of neuropil aggregates in the cortex could also contribute to the selective striatal pathology. This is because the striatum receives numerous projections from the cortex, and corticostriatal transmission is vitally important for the normal function of striatal neurons (*78,79*). The neuropil aggregates in the cortex could affect the corticostriatal transmission and lead to, largely or in part, the dysfunction and cell death observed in the striatum. This idea is particularly appealing because it explains the role of cortical aggregates, and why the distribution of aggregates in the striatum does not correlate well with the selective neuropathology in HD patients' brains. Alternatively, the preferential localization of mutant huntingtin in the nucleus of striatal neurons, as revealed in HD repeat knock-in mice and YAC HD transgenic mice, could affect gene expression or nuclear function before the formation of obvious aggregates.

In summary, the early pathogenic events may be specific to the neuropathology of HD. The early cellular pathology could involve abnormal interactions of mutant huntingtin with other molecules, resulting in altered gene expression by intranuclear huntingtin and impaired synaptic function by neuropil aggregates. Intranuclear aggregates and cell death occur even in non-neuronal cells when mutant huntingtin is overexpressed. On the other hand, neuropil aggregates and intranuclear accumulation of huntingtin precede cell death and are more likely to be associated with HD early cellular pathology in neurons. Further study of the early pathogenesis of HD should also provide insights into how glutamine repeat expansion causes specific neurodegeneration in HD and other glutamine repeat disorders.

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