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Are there multiple pathways in the pathogenesis of Huntington's disease?

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Studies of huntingtin localization in human post-mortem brain offer insights and a framework for basic experiments in the pathogenesis of Huntington's disease. In neurons of cortex and striatum, we identified changes in the cytoplasmic localization of huntingtin including a marked perinuclear accumulation of huntingtin and formation of multivesicular bodies, changes conceivably pointing to an altered handling of huntingtin in neurons. In Huntington's disease, huntingtin also accumulates in aberrant subcellular compartments such as nuclear and neuritic aggregates co-localized with ubiquitin. The site of protein aggregation is polyglutamine-dependent, both in juvenile-onset patients having more aggregates in the nucleus and in adult-onset patients presenting more neuritic aggregates. Studies *in vitro* reveal that the genesis of these aggregates and cell death are tied to cleavage of mutant huntingtin. However, we found that the aggregation of mutant huntingtin can be dissociated from the extent of cell death. Thus properties of mutant huntingtin more subtle than its aggregation, such as its proteolysis and protein interactions that affect vesicle trafficking and nuclear transport, might suffice to cause neurodegeneration in the striatum and cortex. We propose that mutant huntingtin engages multiple pathogenic pathways leading to neuronal death.

Keywords: Huntington's disease; neurodegeneration; basal ganglia; polyglutamine; trinucleotide repeats; protein aggregation

1. INTRODUCTION

(a) *Historical background*

In 1872, George Huntington graphically described the triad of chorea, dementia and autosomal dominant inheritance that marks the disorder now called Huntington's disease (Huntington 1872). He crafted his description from observations made by his father and grandfather, both physicians on Long Island, New York. Alzheimer is credited with showing that small neurons in the striatum disappeared in this illness. Widespread neurodegeneration is appreciated elsewhere in the brain, especially in layers 3, 5 and 6 of the cortex (Vonsattel & DiFiglia 1998). First and foremost, the pathogenic process in Huntington's disease afflicts the medium spiny neuron in the striatum (Aronin *et al.* 1983; Graveland *et al.* 1985). Its genetic cause remained obscure until 1993, when a multi-site collaboration reported that patients with Huntington's disease have an increase in the number of CAG repeats in a gene *IT-15* (interesting transcript number 15), recently named huntingtin (Huntington's Disease Collaborative Research Group 1993). Unaffected subjects had fewer than 35 CAG repeats in tandem, whereas affected individuals had 37 or more of these repeats.

Huntington's disease is a prototype of neurological diseases with lengthened CAG repeats that express proteins with extended polyglutamine sequences; so far, genes with lengthened CAG repeats underlie eight neurological diseases (Bates & Lehrach 1994). It is now clear that the neuropathology of this kind of mutation depends not only on the length of the polyglutamine sequence but also on the parent protein (the context) in which the polyglutamine resides. Recent findings underscore this idea. The introduction of an increased CAG repeat into an otherwise innocuous gene, hypoxanthine phosphoribosyltransferase (HPRT), results in a neurological diathesis in transgenic mice (Ordway *et al.* 1997). We regard this result as fundamental to understanding trinucleotide repeat diseases, because it demonstrates an inherent toxicity of extended polyglutamine sequences. However, the neuropathology was widespread, whereas in each of the trinucleotide-repeat diseases specific brain regions are predominantly affected. Therefore we believe that the polyglutamine sequences confer on the parent protein altered properties culminating in cell-selective disease.

(b) *Thesis*

Our current knowledge of the pathogenesis of Huntington's disease is based on its neuropathology in the juvenile and adult-onset forms as well as insights from transgenic models of Huntington's disease and cultured striatal neurons transfected with mutant huntingtin. Changes in huntingtin localization occur in the Huntington's disease brain and most frequently involve a diffuse nuclear and focal perinuclear accumulation of the protein (Sapp *et al.* 1997). Additionally, in the largest group of Huntington's disease patients, those with adult onset (90–95% of patients) (Snell *et al.* 1993), aggregation of the N-terminal

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995

region of mutant huntingtin in neurites predominate. In the remaining 5–10% of Huntington's disease patients, those with juvenile onset, nuclear inclusions are abundant in the cortex and striatum. The transgenic models of Huntington's disease reveal that expanded polyglutamine repeats serve to target the mutant huntingtin to the nucleus and that small mutant huntingtin fragments are delivered more effectively than large fragments or full-length huntingtin (Davies *et al.* 1997; Laforet *et al.* 1998; Reddy *et al.* 1998). These findings demonstrate, in the human, that cleavage of mutant huntingtin precedes its aggregation and neuropathological consequences. However, even abundant nuclear inclusions do not inevitably cause striatal neurodegeneration (Davies *et al.* 1997). Studies *in vitro* confirm this, with the important caveat that such experiments test short-term, harmful effects of overexpressed mutant huntingtin (Kim *et al.* 1999*a*; Saudou *et al.* 1998). We propose that mutant huntingtin contributes to a number of nuclear and cytoplasmic events that lead to neuronal cell death; the handling of mutant huntingtin in juvenile-onset Huntington's disease probably differs from that in adult onset, resulting in two presentations of the disease.

2. NEURONAL CHANGES IN HUNTINGTON'S DISEASE

(a) *Localization of huntingtin and its implication for function in mammalian brain*

We organized our research plan to determine the localization of wild-type huntingtin and the expression of the mutant protein. The protein coded by the Huntington's disease gene, huntingtin (*ca.* 350 kDa) appears early in the development of the mammalian brain, by day 15 of gestation (Bhide *et al.* 1996). The amount of huntingtin increases in the rodent newborn to achieve adult levels by three weeks. In the adult rodent, wild-type huntingtin resides in the cytoplasm (DiFiglia *et al.* 1995; Sharp *et al.* 1995; Trottier *et al.* 1995). Ultrastructural and biochemical studies in brain point to an association of huntingtin with tubular cisternae and vesicles of the Golgi complex (figure 1*a*), synaptic vesicles (figure 1*b*) (see also DiFiglia *et al.* 1995) and microtubules (Gutenkunst *et al.* 1995; Tukamoto *et al.* 1997), in addition to huntingtin in soluble fractions (DiFiglia *et al.* 1995; Sharp *et al.* 1995). Huntingtin co-distributes with clathrin in differentiating neurons (figure 2*d*) (Kim *et al.* 1999*b*) and decorates clathrin-coated pits and mature clathrin-coated vesicles (figure 2*a,b*) (Velier *et al.* 1998) and uncoated vesicles (figure 2*c*). Co-immunoisolation of huntingtin with synaptophysin in synaptosomes is consistent with the transport of huntingtin to presynaptic sites (DiFiglia *et al.* 1995). In axoplasmic flow studies in peripheral nerve, huntingtin is antero-gradely and retrogradely transported (Block-Galarza *et al.* 1997). On the basis of these results, we speculate that wild-type huntingtin participates in vesicle transport.

Identifying partners for huntingtin has been used to elucidate functions of wild-type huntingtin, as well as possible changes in function of the mutated huntingtin. Huntingtin interacts biochemically with numerous, predominantly cytoplasmic proteins, including huntingtin associated protein 1 (HAP-1) (Li *et al.* 1995), huntingtin interacting protein (HIP-1) (Kalchman *et al.* 1997; Wanker *et al.* 1997), a ubiquitin-conjugating enzyme (Kalchman *et*

Figure 1. Ultrastructural localization of immunoreactive huntingtin in rat cortex. (*a*) Labelling occurs on membranes of tubular cisternae (arrow) and vesicles (ringed arrow) of the Golgi complex (gc). n, nucleus. (*b*) Immunoreactivity in the axon terminal on the right is associated with vesicles (arrow). The labelled terminal forms an asymmetric synapse with a dendritic spine (s). The terminal on the left has no immunoreactivity and also contacts a spine (s). Ab1 antiserum against huntingtin was used. Scale bars, 0.25 µm. (Taken from DiFiglia *et al.* (1995).)

al. 1996), calmodulin (Bao *et al.* 1996), apopain or caspase-3 (Goldberg *et al.* 1996), α-adaptin (Faber *et al.* 1998; Gusella & MacDonald 1998), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Burke *et al.* 1996) and cystathionine β-synthase (Boutell *et al.* 1998). HAP-1 attaches to dynactin (Engelender *et al.* 1997; S. H. Li *et al.* 1998), which contributes to the movement of particles along microtubules; HIP-1 is structurally similar to Sla2P, also implicated in cytoskeletal function in yeast; and adaptin binds to clathrin-coated vesicles. There is also evidence that huntingtin interacts with WW and SH3 protein domains (Faber *et al.* 1998; Sittler *et al.* 1998). Collectively, these studies form the framework that huntingtin might participate, directly or indirectly, in vesicle transport.
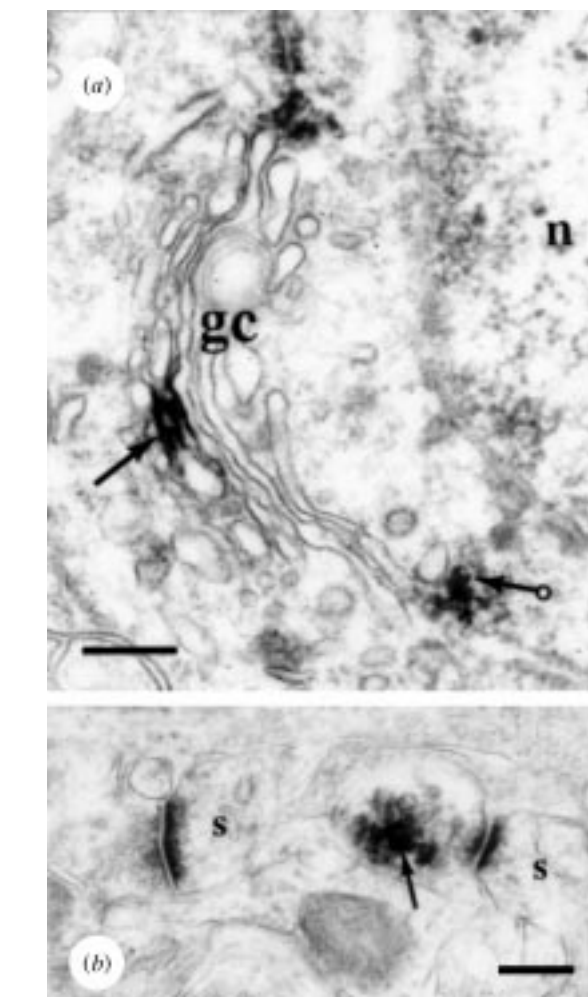
(b) *Expression of mutant huntingtin in Huntington's disease*

Three general mechanisms have been used to explain the pathogenesis of diseases with autosomal dominant
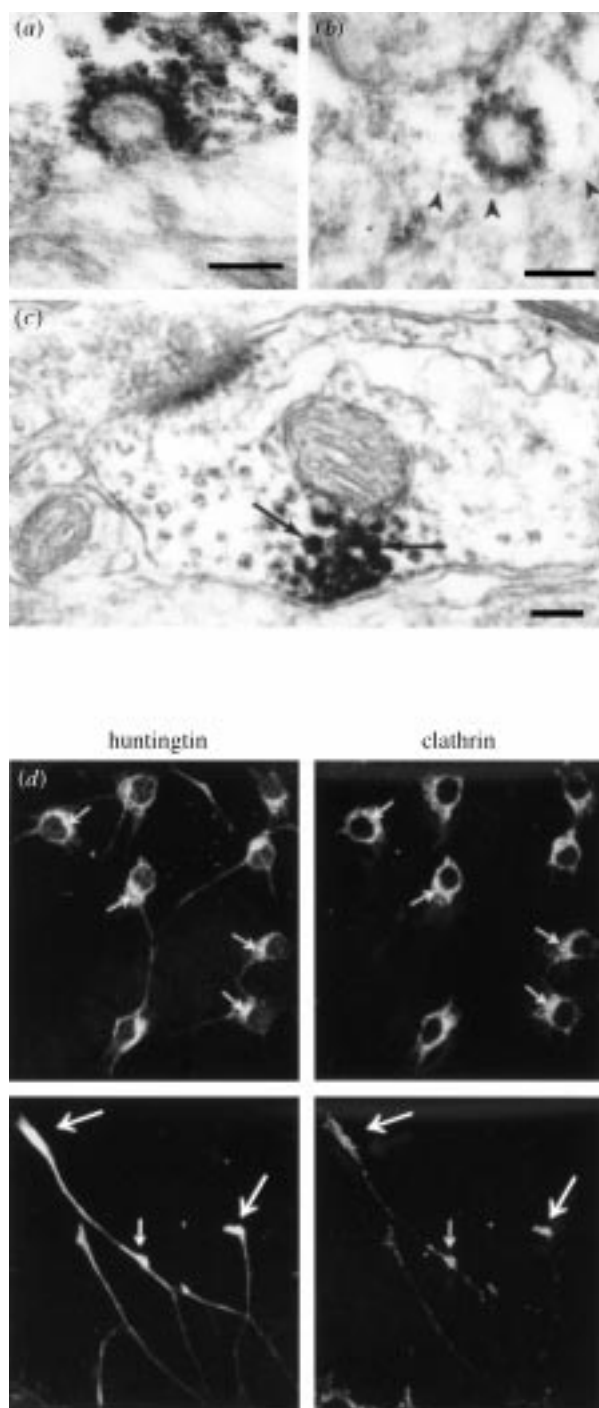
Figure 2. (*a–c*) Electron microscopic localization of immunoreactive huntingtin in dendrites of mouse cortical neurons. Immunoperoxidase labelling is associated with a coated pit (*a*), a coated vesicle (*b*) and with a cluster of vesicles ((*c*), arrows) near the plasma membrane. Staining was performed with anti-huntingtin antiserum Ab585. Scale bars, 0.1 μm. (Taken from Velier *et al.* (1998)). (*d*) Co-localization of huntingtin (with antiserum Ab1) and clathrin in immortalized striatal cells *in vitro*. Both proteins distribute to the same regions of cell bodies (top, arrows) and neurites (bottom, arrows) of differentiated striatal cells. Confocal immunofluorescence microscopy. (Taken from Kim *et al.* (1999*b*).)

inheritance: (i) the mutated gene might not be transcribed, resulting in a decrease in active protein to levels insufficient to perform important cellular functions; (ii) a protein might be translated, but be unable to

perform its activities and block the activities of the wild-type protein; (iii) the protein might change its function or non-specifically render the cell less hardy.

Current information provides evidence that the mutant huntingtin is expressed and maintains its normal activity (which is yet to be defined). We found that mutant huntingtin is expressed throughout the brain in patients with Huntington's disease (Aronin *et al.* 1995). A mechanism for cell death in Huntington's disease needs to consider that unaffected neurons also express the mutant protein. The absence of normal protein does not produce a disease phenotype. Crucially, patients homozygous for Huntington's disease have a phenotype similar to the hemizygous patients (Wexler *et al.* 1987). Collectively, these findings support the idea that the vulnerable neurons should handle the mutant protein differently from protected cells.

(c) *Nuclear inclusions and dystrophic neurites*

We examined the morphology and ultrastructure of neurons in the striatum and cortex in Huntington's disease. Our idea was that study of the human condition would best guide our future experiments. Our findings revealed a complex of subcellular changes, involving cytoplasmic and nuclear structures (DiFiglia *et al.* 1997; Sapp *et al.* 1997), possibly portending a series of molecular aberrations leading to apoptotic neuronal death in Huntington's disease (Portera-Cailliau *et al.* 1995; Butterworth *et al.* 1998). Nuclear changes were readily apparent, but we shall first describe findings in the cytoplasm.

Changes in the cytoplasm pervade the neuropathology in Huntington's disease. With an antiserum directed against an internal epitope of huntingtin (DiFiglia *et al.* 1995), we identified large, immunoreactive granules in the somatodendritic cytoplasm; under electron microscopy, these granules resembled multivesicular bodies, organelles dually involved in retrograde transport and protein degradation (Sapp *et al.* 1997; LaVail & LaVail 1974; Hollenbeck 1993) (figure 3). Huntingtin heavily concentrated in the perinuclear cytoplasm accumulated in part over endosomal–lysosomal organelles and tubulo-vesicular membranes. Some of the full-length protein or large fragments of huntingtin recognized by the antibody might have been destined for the nucleus but was unable to penetrate its borders because it was irreversibly bound to subcompartments of the endoplasmic reticulum.

Another aspect of huntingtin's cytoplasmic localization in the brain with Huntington's disease was revealed by an N-terminally directed antiserum; huntingtin immunoreactivity resided in dystrophic neurites. The dense accumulations of huntingtin overlapped with ubiquitin (DiFiglia *et al.* 1997; Sapp *et al.* 1999) and were embedded in neurofilament-labelled processes (figure 4 *a,b*). Jackson *et al.* (1995) reported dystrophic neurites containing ubiquitin in Huntington's disease. Consisting largely of degenerating axons, the dystrophic neurites with huntingtin marked the striatal and cortical landscape in the adult brain in patients with Huntington's disease and were much more prevalent in adult-onset Huntington's disease than in the juvenile-onset disease (figure 5). A substantial portion of the dystrophic neurites comprised cortical–striatal projections that degenerate early in the disease (Sapp *et al.* 1999) (figure 4*c*).
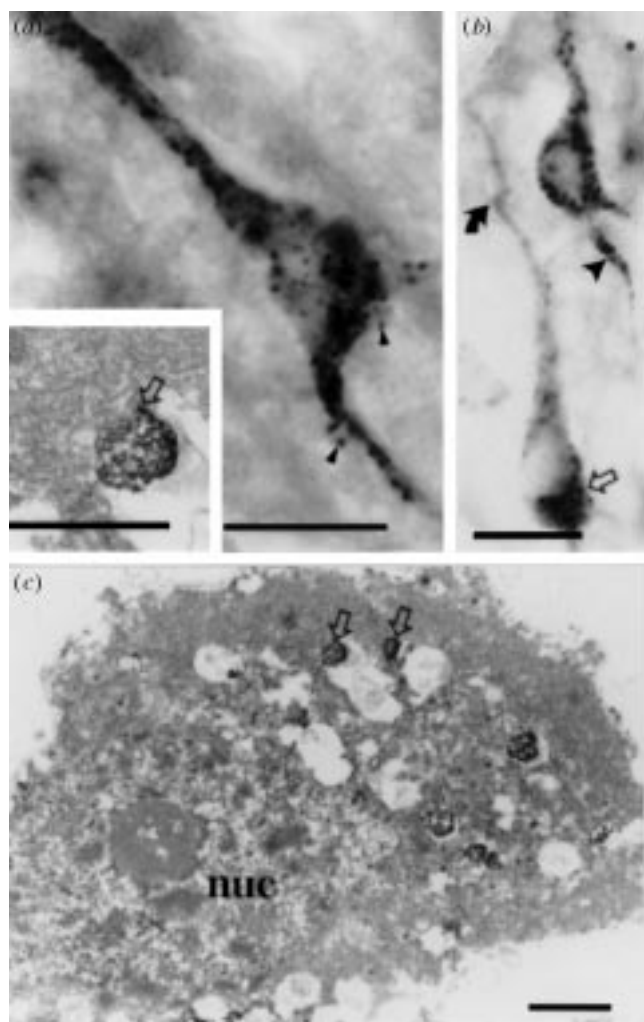
Figure 3. Huntingtin localization in the cortex of a patient with juvenile-onset Huntington's disease. (*a*) Immunoreactive granules fill the somatodendritic cytoplasm (arrowheads). (*b*) Huntingtin staining is concentrated in the perinuclear cytoplasm of the soma on the left (open arrow) and in punctate granules in the cell on the right. The curved arrow shows the retraction of an apical dendrite and the arrowhead identifies a possible axon initial segment. Staining was performed with anti-huntingtin antiserum Ab585. (*c*) Electron micrograph of pyramidal neuron; huntingtin-labelled granules (arrows) appear throughout the cytoplasm. nuc, nucleus. The inset in (*a*) shows a higher magnification of a granule that resembles a multivesicular body. The scale bars in (*a*–*c*) are 25 µm and that in the inset is 5 µm. (Taken from Sapp *et al.* (1997).)

Figure 4. N-terminal mutant huntingtin in dystrophic axons in the Huntington's disease brain. (*a*) Double-label immunofluorescence shows a huntingtin immunoreactive dystrophic neurite (arrow) positioned within a neurofilament-labelled axonal process in the cortex. (*b*) Co-localization of huntingtin with ubiquitin in a cortical dystrophic neurite. (*c*) Nascent dystrophic axons traverse the striatal neuropil of adult-onset patient with low-grade striatal pathology. Arrow identifies abnormal swelling. ((*a*,*b*) Taken from DiFiglia *et al.* (1997); (*c*) from Sapp *et al.* (1999).)

Wild-type huntingtin is absent from, or rare in, the nucleus in neurons in control brains, on the basis of most studies (DiFiglia *et al.* 1995; Sharp *et al.* 1995; Trottier *et al.* 1995), although there is a report of nuclear localization with appropriate biochemical correlates (De Rooij *et al.* 1996). In post-mortem human striatum in Huntington's disease, we found moderately intense, diffuse nuclear localization of huntingtin detected with the use of anti-serum against an internal epitope of the protein (Sapp *et al.* 1997). The N-terminal antiserum unmasked huntingtin in dense intranuclear inclusions in brains with juvenile-onset Huntington's disease (figure 6), and to a smaller extent in adult-onset Huntington's disease (DiFiglia *et al.*
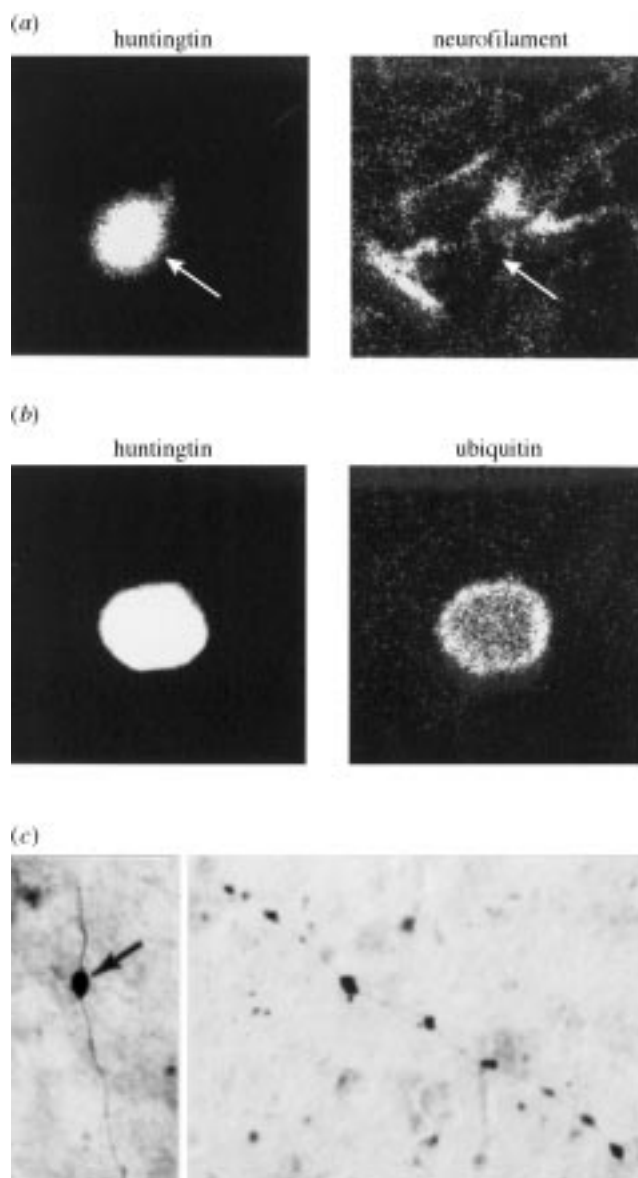
1997). The nuclear inclusions were dispersed in neurons of cortex and striatum (figure 6), regions of most active disease, but not in globus pallidus or cerebellum (figure 6*e*,*f*), which are largely unaffected. Like the dystrophic neurites, the nuclear inclusions contained ubiquitin (figure 6*a*). Ultrastructural review revealed granular and filamentous configurations (figure 6*g*). Contemporaneously, nuclear inclusions were identified in other CAG trinucleotide repeat diseases (Becher *et al.* 1998; Holmberg *et al.* 1998; M. Li *et al.* 1998; Merry *et al.* 1998; Paulson *et al.* 1997).

The formation of nuclear inclusions was polyglutamine-dependent and was more frequent with larger
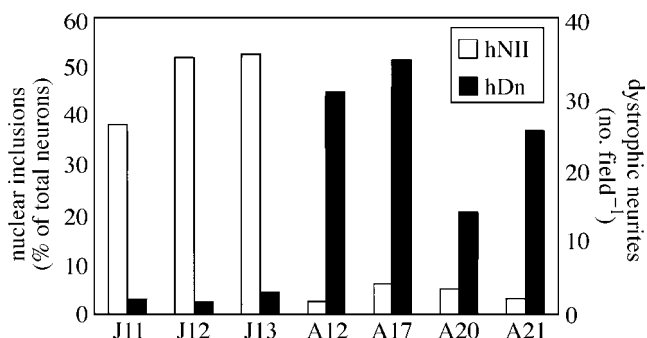
Figure 5. Frequency of nuclear inclusions and dystrophic neurites in patients with Huntington's disease with juvenile onset (J11, J12, J13) and adult onset (A12, A17, A20, A21). Nuclear inclusions are more frequent in juveniles, and dystrophic neurites are more abundant in adults. (Taken from DiFiglia *et al.* (1997).)

expansions; 30–50% of neurons in the cortex in juvenile-onset Huntington's disease contained nuclear inclusions, compared with *ca.* 3–6% of cortical neurons in the adult-onset disease (DiFiglia *et al.* 1997; Vonsattel & DiFiglia 1998) (figure 5). This finding was not related to the neuropathological grade of striatal degeneration (DiFiglia *et al.* 1997). In the adult-onset patients with low-grade neuropathology (grades 1 and 2), nuclear inclusions were absent from, or rare in, the striatum when dystrophic neurites had formed (Sapp *et al.* 1999).

How does huntingtin enter the nucleus? The large size of huntingtin would predict its nuclear entry either by active transport or by its being processed to form smaller fragments. Measurement of huntingtin immunoreactivity in isolated nuclei demonstrated that much of the huntingtin in the nuclei consisted of N-terminal fragments of lower molecular mass, in the 20–40 kDa range (DiFiglia *et al.* 1997) (it should be noted that approximating the molecular mass of proteins containing lengthy polyglutamine sequences is risky). Proteins of this approximate size could enter the nucleus passively (Alpert *et al.* 1994). Smaller, cleaved products of mutant huntingtin were shown to enter the nucleus preferentially (Martindale *et al.* 1998; Hackam *et al.* 1998). Detloff and co-workers showed that highly extended polyglutamine sequences can promote the nuclear entry of an otherwise cytoplasmic protein (Ordway *et al.* 1997). We found full-length huntingtin in nuclear extracts of brain with Huntington's disease. Larger proteins have access to the nucleus, but enter passively at a low rate, or enter by facilitated transport or active import. We contend that over a time frame of years to decades, with the lengthened polyglutamine sequences as facilitator, small and large huntingtin fragments and full-length protein have an opportunity to gain access to the nucleus.

3. IS NUCLEAR AGGREGATION OF HUNTINGTIN NECESSARY OR SUFFICIENT TO TRIGGER CELL DEATH IN STRIATAL NEURONS?

(a) *Lessons from transgenic models of Huntington's disease*

Several laboratories have generated transgenic mice expressing either an N-terminal fragment or a full-length mutant huntingtin (Mangiarini *et al.* 1996; Reddy *et al.*
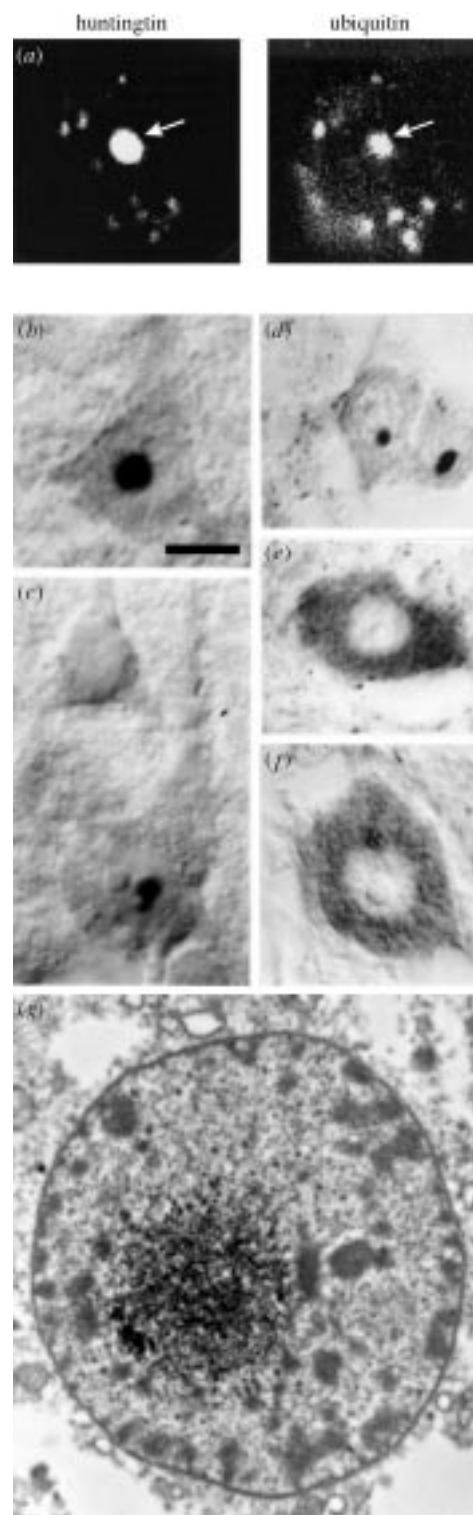

Figure 6. Nuclear inclusions formed by N-terminal mutant huntingtin in the brain with Huntington's disease. (*a*) Immunofluorescence shows co-localization of huntingtin with ubiquitin (arrows) in the same inclusion of a cortical pyramidal cell. (*b,c*) Immunoperoxidase labelling shows a nuclear inclusion distinct from the adjacent unlabelled nucleolus in cortical pyramidal neurons. (*d*) Two medium-sized striatal neurons have nuclear inclusions. (*e,f*) Neurons in the globus pallidus (*e*) and cerebellum (*f*) are devoid of nuclear inclusions and show labelling in the cytoplasm. (*g*) Electron micrograph showing immunoperoxidase labelling associated with granules and filaments within an inclusion. The scale bar in (*b*) is 10 μm. (Taken from DiFiglia *et al.* (1997).)

1998; Cha *et al.*, this issue; Davies *et al.*, this issue; Reddy *et al.*, this issue). Differences between the transgenic mice include the length of the polyglutamine expansion, the size of the huntingtin, the promoter and the background strain. The initial transgenic mice with Huntington's disease (as previously described) used a human huntingtin gene promoter to express mutant huntingtin with an expanded polyglutamine repeat (115–150 repeats) and a small huntingtin N-terminal fragment (exon 1, 69 residues) in a CBL/6 mouse strain. This transgenic strain was very instructive. Despite extensive nuclear inclusions, striatal cell loss was absent or minimal. The animals succumbed within three months to general inanition, although recent evidence implicates diabetes mellitus (with hypoinsulinaemia) as a cause (Hurlbert *et al.* 1998). Detloff and co-workers created transgenic mice expressing HPRT with a stretch of 146 glutamine residues. HPRT is ordinarily localized in the cytoplasm, but these animals showed abundant nuclear inclusions throughout the brain. No cell death was observed. The animals were short-lived and exhibited abundant seizures. These studies indicate that small proteins with expanded polyglutamine sequences are targeted to neuronal nuclei and accumulate in sufficient abundance to form large aggregates, detected as inclusions. Transgenic animals harbouring expanded polyglutamine stretches in the context of small proteins manifest phenotypic changes consistent with generalized brain dysfunction. However, the presence of inclusions does not predict cell-specific or region-specific neuro-degeneration under these conditions.

Tagle and co-workers (Reddy *et al.* 1998, this issue) and our own laboratory (Laforet *et al.* 1998) made transgenic models of Huntington's disease expressing a substantially larger mutant huntingtin than in the initial transgenic mice with Huntington's disease. Our transgenic mice expressed a 46 or 100 CAG repeat (one base change at position 28 CAG) in the first 3221 bases of huntingtin complementary DNA (cDNA). The host strain was SJL/B6 F1 hybrid. Many animals exhibited motor deficits, lost *ca.* 20% of striatal cells and lived beyond one year (Laforet *et al.* 1998; G. A. Laforet, M. DiFiglia and N. Aronin, unpublished data). Nuclear inclusions were apparent but not abundant in the striatum of normal, affected mice in lineages with 46 or 100 glutamine repeats. Tagle and co-workers generated transgenic mice with 16, 48 and 89 glutamine repeats in a full-length huntingtin in FVB/N murine strain. Mutant huntingtin expression varied, from nearly equivalent to native protein to about fivefold greater. Nuclear inclusions were detected, but not abundantly, and the animals had a predictable 20% cell loss in the striatum and cortex (Reddy *et al.* 1998, this issue).

These studies *in vivo* reveal that the size of the huntingtin protein substantially influences the longevity of the transgenic mice with Huntington's disease and also the presence of striatal cell loss. Neither the background strain of the mice nor the abundance of nuclear inclusions accounts for neurodegeneration.

(b) *Revelations in the short-term: studies in neurons and other cells in culture*

Experiments in cell culture have provided important clues to the role of inclusions of huntingtin in the patho-genesis of cell death. We studied effects of mutant huntingtin expression in cultured hybrid striatal, medium-sized neurons, generated from clonal mouse foetal striatal-neuroblastoma fusions (Kim *et al.* 1999a). These cells maintain many fundamental characteristics of striatal spiny neurons, which are the initial targets of neurodegeneration in Huntington's disease (Wainwright *et al.* 1995). Constructs containing 18, 46 or 100 CAG repeats in either a full-length huntingtin cDNA or a large fragment of huntingtin cDNA (1–3321 bases) were trans-fected into the clonal striatal neurons. The cDNAs were intact or modified as N-terminal FLAG–huntingtin fusion proteins or huntingtin–green fluorescent protein (GFP) fusion proteins; huntingtin and FLAG were detected by immunohistochemistry and GFP was viewed by fluorescence (Kim *et al.* 1999a). Full-length and 3 kb mutant huntingtin cDNAs (irrespective of molecular tags) produced nuclear inclusions. Tranfection with the 100 CAG repeat huntingtin cDNA resulted in a greater number of inclusions than with the 46 repeat cDNA; the 18 repeat produced none. Therefore the clonal striatal neurons recapitulated the polyglutamine-dependent formation of inclusions seen in the patient brain in Huntington's disease.

We have found that transfection of partial or full-length mutant huntingtin cDNAs into clonal striatal cells generated N-terminal fragments; the sizes varied between 70 and 100 kDa (Kim *et al.* 1999a). Because huntingtin can be a target of caspase cleavage (Goldberg *et al.* 1996), we introduced caspase inhibitors into the cultures of transfected cells. With the caveats that caspase inhibitors lack true specificity and probably affect separate compo-nents of apoptotic cascades, we considered the inhibitors primarily as tools to block huntingtin degradation.

The caspase inhibitor Z-DEVD-FMK (Enzyme Systems) prevented inclusion formation after transfection with mutant huntingtin cDNAs. No abatement in cell death was observed (Kim *et al.* 1999a). An implication of this result is that huntingtin nuclear inclusions are not necessary for mutant huntingtin to induce striatal cell survival. In contrast, we found that the general caspase inhibitor Z-VAD-FMK (Enzyme Systems) did not alter mutant huntingtin nuclear inclusions, but (surprisingly) did improve the survival of the transfected clonal striatal cells. In a separate model, Saudou *et al.* (1998) reported that entry of an N-terminal fragment of mutant huntingtin (171 residues, 68 glutamine repeats) into the nucleus reduced survival of the transfected primary rat striatal neurons. By placing a nuclear export signal in the mutant huntingtin, the authors discovered that accumulation of the transfected mutant huntingtin was abrogated and cell survival was unaffected. They found that inclusion forma-tion was not requisite for cell death, but that delivery of a mutant huntingtin fragment to the nucleus was needed to induce apoptosis. Similarly, in neuroblastoma cell lines stably expressing mutant huntingtin, Lunkes & Mandell (1998; Lunkes *et al.*, this issue) found that apoptosis was increased by the expression of mutant huntingtin but was not tightly correlated with the formation of nuclear and cytoplasmic inclusions.

The above studies *in vitro* suggest that nuclear inclusions are not necessary or sufficient to predict striatal cell death, which is reminiscent of the neuropathology seen in

adult-onset Huntington's disease. Other compartments that accumulate huntingtin might be more critical for cell death. In striatal hybrid neurons, we found that cell death (marked by significant cell shrinkage) occurred when N-terminal huntingtin fragments of wild-type or mutant proteins accumulated into perinuclear vacuoles (Kim *et al.* 1999*a*), not unlike the perinuclear huntingtin accumulation associated with degenerating neurons in the brain with Huntington's disease (Sapp *et al.* 1997). That N-terminal fragments of mutant huntingtin can influence cellular toxicity has support from studies in the laboratories of Hayden (Hackam *et al.* 1998) and Ross (Cooper *et al.* 1998), who showed that introducing N-terminal fragments of mutant huntingtin into neuroblastoma cell sensitized the cells to apoptosis. We found that a blockade of one N-terminal huntingtin product by caspase inhibition was correlated with increased cell survival (Kim *et al.* 1999*a*). N-terminal products of either wild-type or mutant huntingtin are toxic at high levels in non-striatal and striatal cells (Hackam *et al.* 1998; Kim *et al.* 1999*a*). How, then, can N-terminal mutant huntingtin fragments be selectively disruptive to cellular function? Insight comes from crucial studies in which full-length huntingtin was overexpressed in cultured cells (Lunkes & Mandel 1998; Lunkes *et al.*, this issue; Kim *et al.* 1999*a*). Under these conditions, N-terminal fragments accumulated only when the protein contained an expanded polyglutamine tract.

4. MULTIPLE PATHOGENIC MECHANISMS IN HUNTINGTON'S DISEASE

We present evidence that mutant huntingtin accumulates in multiple subcellular compartments in selected neurons in Huntington's disease and is handled differently in the juvenile-onset patient compared with the adult-onset patient. Available information points to two general pathways leading to cell death, with an emphasis on nuclear disruption in juvenile-onset Huntington's disease and the axon as a major site of pathology in adult-onset Huntington's disease. In general, the larger is the CAG repeat, the more likely it is that mutant huntingtin will be targeted to the nucleus in addition to its accumulation in the cytoplasm. Neuronal death is exacerbated by the accumulation of N-terminal fragments of huntingtin in either the nucleus or cytoplasm but does not depend on the presence of nuclear or cytoplasmic inclusions. The N-terminal region of huntingtin is cleaved from the full-length protein and robustly amasses in cells when the polyglutamine tract is expanded. Mutant huntingtin's N-terminal domain interacts abnormally with cytoplasmic proteins involved in vesicle trafficking (HIP1, HAP1, GAPDH and SH3GL3). The early appearance of dystrophic axons enriched in N-terminal mutant huntingtin in adult-onset patients conceivably coincides with disturbances of vesicle membrane transport in these axons. Huntingtin also interacts at its N-terminus with resident nuclear proteins (Faber *et al.* 1998). Alterations in nuclear function caused by targeting of the cleaved or fully intact protein to the nucleus, with the tendency of huntingtin to form filaments (Perutz *et al.* 1994; Scherzinger *et al.* 1997), are especially pronounced in patients with juvenile-onset Huntingtons' disease and

even pre-empt changes in the cytoplasm. We believe that a single pathogenic event causing Huntington's disease might prove elusive.

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