Molecular Neurobiology

ISSN0893-7648/01/23(1): 21–51/$17.75

Modeling Huntington's Disease in Cells, Flies, and Mice

Simonetta Sipione and Elena Cattaneo*

Department of Pharmacological Sciences, University of Milano, Center of Excellence on Neurodegenerative Diseases, Via Balzaretti 9, 20133 Milano, Italy

Abstract

A milestone in Huntington's disease (HD) research is represented by the identification of the causative gene. With the genetics at hand, a series of transgenic cellular and animal models has been developed, which has greatly contributed to understanding of HD. All these models are described in this review, and are compared to each other, along with the information they have generated. Although the mechanism by which progressive loss of striatal neurons occurs in HD remains uncertain, hypotheses on mutant huntingtin toxicity involve impaired vescicular trafficking, transcriptional dysregulation, and/or activation of apoptotic pathways. The development of inducible HD mice has shown that neurodegeneration in HD may be at least partially blocked. Although traditionally considered a "gain-of-function" disease, the recent finding that normal huntingtin has an important role in neuronal survival suggests that loss of function of the normal protein might contribute to HD as well, also discloseing new perspectives on the therapeutical approach to the pathology.

Index Entries: Huntingtin; models; caspases; Huntington's Disease; apoptosis.

Introduction

Huntington's disease (HD) is an inherited neurodegenerative disease, mainly affecting striatal and cortical neurons. The cause of the disease has been identified in the expansion of a stretch of CAG repeats in the coding region of the gene for huntingtin, a 348-kDa protein whose function has only begun to be elucidated. The disease, which is greatly disabling, progressive, and leading to death, has a high prevalence in the Caucasian population, affecting 4–10/100,000 individuals.

After the gene responsible for HD was identified in 1993, great progress has been made in understanding of pathology, thanks to the considerable number of more precise animal and cellular transgenic models that have been generated. However, despite a decade of intense research in the field, crucial questions remain: why are selected subpopulations of neurons affected? What are normal and pathological

*Author to whom all correspondence and reprint requests should be addressed. E-mail: cattaneo@unimi.it.

huntingtin doing in those cells? What strategies should be developed to fight the disease? Every month new data and information on the genetics, biology, biochemistry, and physiology of HD appear as pieces of a complex puzzle. The authors try to put them together, to give an overview of the state-of-the-art in this field, summarizing, at the same time, the animal and cellular models that help in composing this puzzle.

Clinical and Neuropathological Features of HD

HD is characterized by motor, cognitive, and behavioral dysfunction. Motor symptoms are the most peculiar feature of the disease, although the pattern of symptoms and their progression may vary among affected individuals. Patients usually show involuntary choreic movements and impaired control of voluntary motor functions. With disease progression, rigidity, bradykinesia, and dystonia usually supersede chorea. There is general agreement in considering the disease onset as the time when motor symptoms begin, which is, on an average, at the age of 35–40 yr. Yet, as symptoms begin insidiously, the exact assessment of the disease onset is sometimes problematic *(1–4).*

Recently, deficits in the control of skilled arm movements, and in the ability to accurately point at targets, have been identified in presymptomatic patients, up to 7 yr before the predicted onset of clinical symptoms *(5).* Hence, the question arises as to whether HD is really a late-onset disease, or rather does it become evident only when symptomatology has already progressed to a late stage. In the latter case, therapy, used when the disease is not yet advanced, would surely produce the highest benefits.

Weight loss is another consistent feature of HD, but the underlying cause is not clear *(6–9).*

Long expansion of the CAG repeats results in juvenile onset of HD, and a symptomatology that is usually more severe and often characterized by different hallmarks than adulthood-onset HD, such as rigidity, tremor, and epileptic seizures *(10,11).*

HD symptomatology correlates well with the neuropathology, the most striking feature (but not the only one) being a dramatic loss of neurons and astrogliosis in the striatum *(12),* with a reduction up to 60% of the striatal volume. Gamma-aminobutyric acid-ergic, medium-sized, spiny neurons, projecting to substantia nigra and globus pallidus, are selectively affected, with a preferential loss of the neuronal subpopulation expressing enkephalin and D2 receptor, at least in the early stages of HD *(13–15).*

Cortical atrophy is another prominent feature in HD neuropathology, with the more pronounced degeneration occurring in layers III, V, and VI *(16,17),* though brain regions may show signs of the disease *(12,18).*

Although HD mainly affects the central nervous system (CNS), minor effects on other systems may be present. Indeed, abnormal skeletal muscle energy metabolism has been observed, caused by a deficit in mitochondrial oxidative metabolism *(19).*

Genetics of HD

HD is inherited as a typical autosomal-dominant feature. The HD locus was mapped on 4p16.3 chromosome in 1983 *(20),* but, only 10 yr later, the gene responsible for HD was identified *(21).* As mentioned, a polymorphic CAG repeat (9–36 repeats) is present in exon 1, and it is expanded (above 36 repeats) in patients with HD *(22,23).*

An inverse correlation exists between the number of CAG repeats and age of onset *(24,25),* with cases of juvenile onset HD characterized by 100 or more repeats. However, the CAG repeat number only explains 50% of the variance in age of onset *(4,26).* In search for modulating factors, variations in the kainate-specific glutamate receptor GluR6 genotype have been shown to account for some of the fluctuations in the age of onset of HD not

accounted for by CAG repeat length *(27).* Genetic polymorphisms adjacent to the CAG repeats have been suggested to influence age of onset, as well *(28–30).* Indeed, patients carrying the Δ2642 glutamic acid polymorphism (a deletion of three nucleotides encoding for Glu at codon positions 2642–2645) develop the disease earlier than predicted by the CAG number in their HD gene *(28,31).* This suggests that the action of mutant huntingtin may be influenced not only by the CAG expansion, but also by possible *cis*-acting elements, or by the normal allele itself *(32,33).* So far, no differences in symptomatology, age of onset, and disease progression have been reported between heterozygous and homozygous HD patients, but the small number of homozygous cases analyzed and flaws in statistical approaches in earlier studies may have been misleading *(34).* In light of some recent findings on the normal role of huntingtin, re-evaluation of all these data becomes necessary.

Expanded CAG repeats are preferentially associated with seven downstream CCG repeats *(35,36).* Reduced prevalence of HD is correlated with higher frequency of alleles with shorter CCG stretch in Finland, Japan, China and among African Blacks *(37).*

A well-known phenomenon associated with HD is the genetic anticipation, i.e., individuals inheriting the disease from the father bear a longer CAG stretch and, consequently, develop the disease at a younger age *(26).* The molecular mechanism responsible for repeat expansion in HD is not yet clear. Increased triplet number may derive from replication slippage *(38)* and the large number of mitotic divisions during spermatogenesis may account for the expansion of the CAG stretch. Somatic instability and tissue mosaicism of the CAG repeat number in HD patients have also been reported *(39).* The highest variability was found in cortical and striatal neurons, and in spermatozoa. However, the possibility that increased polyglutamine (polyQ) load may contribute to the pattern of selective neuronal cell death in HD remains controversial. More recently, Kennedy and Shelbourne *(40)* have reported on a dramatic increase of the CAG expansion in striatal cells from old HD knock-in mice. Tissue mosaicism and repeat instability have also been observed in somatic cells of HD transgenic mice *(41).* Following the observation that no instability was present in an HD transgenic male mouse, in which the transgene was not expressed, Mangiarini et al. *(41)* suggested that repeat instability may be related to gene transcription, rather than DNA replication. The mechanism underlying triplet repeats expansion in somatic cells, and its role in HD pathogenesis, remain to be elucidated.

Huntingtin Expression and Regulation

The presence of a CAG expansion in the HD gene does not significantly alter regional and cellular expression of huntingtin protein *(39,42,43).* Both wild-type and mutant protein are widespread in the CNS and peripheral tissues *(44–48),* and studies conducted so far, although sometimes discordant, failed to demonstrate any correlation between huntingtin expression and neuronal vulnerability *(49,50).* However, one study has recently called into question huntingtin expression in the striatal neurons (shown to be very low), demonstrating, instead, that corticostriatal neurons are rich in huntingtin protein and mRNA *(49).* These data suggest that re-evaluation of the distribution and expression levels of normal and mutant huntingtin, in the cortex and striatum of HD brains, may be necessary, since distinction between normal and mutant protein may have been set with difficulty. As a matter of fact, Ona et al. *(51)* recently reported that not only are levels of endogenous full-length huntingtin low in the brain of transgenic mice carrying an N-terminal portion of the mutant protein (R6/2 mice), but that the majority of wild-type protein is cleaved, generating a smaller fragment. This finding suggests that loss of normal huntingtin function may occur in these mice.

Very little is known about regulation of huntingtin expression. The HD gene encodes two mRNA transcripts of different molecular weight (10 and 13.7 kb), resulting from two different polyadenylation signals *(52).* The larger mRNA is the predominant transcript in human brain; the shorter one is particularly enriched in testis *(53–55).*

The promoters of the human HD gene *(56)* and the murine homologous *Hdh* gene (from both mouse and rat) have been isolated and characterized *(56,57).* They all present a high GC content and lack of typical TATA and CAAT *cis*-elements, suggesting that they are "housekeeping" genes. A putative AP2-binding site and numerous potential Sp1-binding sites are present, along with binding sites for other transcription factors that may be acting during development *(57).* In the rat promoter, a binding site for SRY, a transcription factor essential for testis development *(58,59),* has been identified, as well *(57).*

Modulation of huntingtin levels occurs in a cell-specific manner upon phorbol esters exposure *(60,61),* and a significant upregulation of huntingtin in astrocytes of the arcuate nucleus of the hypothalamus of lactating rats has been reported *(62).* Huntingtin upregulation also occurs upon desferroxamine mesylate administration, indicating that its expression may be regulated by iron *(63).*

Animal Models of HD

In the pregenomic era of HD research excitotoxic lesions by intrastriatal injections of quinolinic acid *(64–67)* and metabolic impairment by administration of 3-nitropropionic acid *(68)* have been valuable and unique means for reproducing in animals a pattern of striatal neurodegeneration resembling human HD.

Although we are still learning from these toxin-induced models, the development of transgenic HD animals has had a tremendous impact in the field, allowing for the more precise reproduction of the pathology, and for the analysis of disease progression. Until now, several genetic models have been generated, each of them having strengths and weaknesses. Probably no single model can yet recapitulate the human pathology entirely (maybe no animal model can), however a complementary analysis of data coming from different models may provide a global picture closer to the human pathology.

Knockout of Huntingtin Gene

Targeted disruption of the huntingtin gene in mouse was performed to analyze whether a HD-like phenotype could be generated. *Hdh* (the murine homolog of the human HD gene) knockout invariably led to embryonic lethality by embryonic day 8.5 *(69–71)*, and this was accompained by a remarkable increase in the rate of apoptosis in the embryonic ectoderm *(69,71).* Experiments on chimeric embryos, produced by injection of *Hdh*-null ES cells ($Hdh^{-/-}$) into wild-type blastocysts ($Hdh^{+/+}$), suggested that embryonic lethality, in the absence of huntingtin, was possibly caused by an impairment of the nutritive function of extraembryonic tissues *(72).* Besides demonstrating an essential role for huntingtin during development, these studies also showed that a simple loss of function of huntingtin protein, in the presence of an expanded CAG repeat, could not account, by itself, for the HD pathology. The lack of a HD-like phenotype in knockout mice has always been interpreted as a proof of a gain-of-function activity of the polyQ expansion in HD. The same conclusion was drawn from data on heterozygous *Hdh* knockout mice ($Hdh^{+/-}$) which showed a normal phenotype in two different models *(69,71).* However, in a third model (in which knockout mice still produced a putative truncated N-terminal fragment of the protein), heterozygous mice showed increased motor activity, cognitive deficits, neuronal loss and degeneration in the subthalamic nucleus and globus pallidus *(70).* To explain discrepancies with the

previous two models, it was suggested that the putative truncated protein produced by the targeted allele might exert a dominant effect in the heterozygous mice, therefore generating a phenotype, and that huntingtin may play a key role in normal functioning of the basal ganglia *(73).* However, lack of endogenous huntingtin does not prevent homozygous knockout ES cells from differentiating into neuronal-like cells that can establish functional synapses and express functional voltage- and neurotransmitter-gated ion channels *(74).*

Other features could be modulated by huntingtin in postsynaptic neurons. One of these is the neuronal response to N-methyl-D-aspartate (NMDA)-evoked currents, which have a higher amplitude in knockout neuronal-like cells, compared to wild-type neurons *(74).* Increased sensitivity to NMDA has been also observed in transgenic *(75)* and knock-in models for mutant huntingtin *(76),* suggesting that loss of at least some of the functions of huntingtin may occur in the presence of the expanded CAG. Huntingtin knockout ES cells can also normally differentiate into hematopoietic progenitors *(77),* but, in the presence of hematopoietic cytokines, heterozygous and homozygous knockout cells show a gene-dosage-dependent decrease in proliferation and survival. Finally, if expression of huntingtin is reduced by >50%, abnormal brain development and perinatal lethality are observed *(78),* indicating that huntingtin may play a role in regulating the balance between proliferation and death.

The essential role for huntingtin during embryonic development long precluded assessment of its function in the adult brain. The problem has recently been circumvented by Dragatsis et al. *(79),* who have developed a conditional knockout model of huntingtin. In this model, the Cre-loxP site-specific recombination system has been used to generate mice in which the *Cre* gene is expressed under a neuronal specific promoter (α-calcium/calmodulin-dependent protein kinase II). Conditional knockout animals show progressively more severe motor deficits, which are evident by the age of 10–12 mo, and have a reduced life-span. In the brain, tissue degeneration occurs in the striatum and cortex, and, in older mice, it is accompanied by degeneration of axon fibers and more limited, but evident, apoptosis. These features are similar to those reported in currently used transgenic models of HD, suggesting that loss of huntingtin function may greatly contribute to the pathological phenotype.

As a result of Cre-mediated recombination and reduced *Hdh* expression in testis, atrophy of seminiferous tubules and sterility were present in the conditional knockout animals. Among the non-CNS tissues, huntingtin is most highly expressed in testis *(44,54),* where it may play a role in the process of sperm maturation *(79).* In agreement with this hypothesis is the finding that upregulation of huntingtin occurs, in vitro, in rat male germ cells co-cultivated with Sertoli cells *(55),* and that YAC transgenic mice bearing mutant huntingtin, but lacking the endogenous wild-type protein, show massive spermatocyte loss and testicular atrophy *(80).*

Transgenic Mice Expressing N-Terminal Portions of Huntingtin

The first transgenic model of HD was developed by Mangiarini et al. *(81),* who introduced the human huntingtin exon-1 with 115–150 CAG repeats, along with its own promoter, in mice. These mice (the most widely used mice line being R6/2) show a progressive neurological phenotype that includes involuntary stereotypic movements, tremors and epileptic seizures, weight loss, and premature death. Neurotransmitter receptors distribution is altered at least 1 mo before the onset of motor symptoms *(82),* in coincidence with a reduction of the corresponding mRNA levels *(83).* Besides the neurological phenotype, metabolic abnormalities occur in adipose tissue, where an impaired regulatory control of lipolysis and leptin release may be responsible for the higher per-

centage of body fat observed in young animals, and the later wasting that accompanies the pathology *(84).* The R6/2 mice frequently develop an insulin-responsive diabetes *(85),* perhaps as a consequence of polyQ amyloid accumulation in pancreatic islets *(86).* Whether this is an artifactual consequence of the transgenic model, or rather the reproduction, in the mouse model, of a feature of the human pathology, is not yet clear. In fact, increased frequency of diabetes in HD patients, compared with the normal population, has been reported *(87),* although it is not known whether this is related to huntingtin aggregates. In the R6/2 mice, huntingtin aggregates are found in the nucleus of neurons prior to the onset of symptoms *(88),* as well as in skeletal muscle cells, heart, liver, and pancreas *(86).* A similar neuropathological phenotype was also obtained in a transgenic mouse model expressing a slightly larger N-terminal fragment (N-171) of human huntingtin containing 82Q *(89).*

New perspectives and hopes for the possibility for curing HD have been recently disclosed by the surprising data obtained in an inducible HD exon-1 mouse model *(90).* In these mice, expression of mutant huntingtin is turned off at will via tetracycline-induced inactivation of a transcriptional transactivator *(91).* Mice expressing HD exon-1 with 94 CAG repeats showed the typical behavior and progressive neurodegenerative features seen in previous models, including limb clasping upon suspension by tail, reduced size of striatum, reactive gliosis, and a decrease in D1 receptors. However, upon suppression of mutant huntingtin expression by injection of the tetracycline homolog, doxycycline, the progressive striatal degeneration was arrested. Even more surprisingly, huntingtin aggregates disappeared and motor behavior improved significantly, in some cases reversing to the levels of control mice. These data indicate that progression of HD disease requires the constant production of mutant huntingtin, and, more importantly, that neurons not only retain the ability to dismantle and metabolize aggregates, but also to reverse a pathological phenotype *(92,93).* Whether this may be true for humans as well (and for the full-length mutant protein) is not yet known, but it certainly provides hope for a treatment of patients with already advanced HD symptoms.

Symptoms and neuropathology reminiscent of the HD exon-1 transgenic mice have been also reproduced in transgenic mice, by expressing a long polyQ stretch inserted into the hypoxanthine phosphoribosyltransferase protein (Hprt), an ubiquitous protein, unrelated to HD, that normally lacks a polyglutamine domain *(94).* This finding might be considered proof that the polyQ stretch is enough, by itself, to determine HD, by means of a gain-of-function mechanism. Yet, several other neurodegenerative diseases are caused by a polyQ expansion in genes of known or unknown function *(95),* and a distinctive pattern of neurodegeneration is observed in each of them, which suggests an important role for the full-length protein in determining the overall effects of the mutation and the cell-specificity of the neurodegeneration. Exon-1 of huntingtin represents only 3% of the full-length protein, and, consequently, transgenic models based on expression of the N-terminal portion of huntingtin may not reproduce the same cell specificity and temporal evolution of the degeneration seen in HD. Indeed, these models have a shorter life-span and an earlier onset of motor symptoms, compared to transgenic animals expressing full-length huntingtin (Table 1). Moreover, R6/2 mice appear to display pathological features not shared by human patients, such as aggregate accumulation and generalized atrophy in several extra-CNS organs *(86).*

Full-Length Huntingtin Transgenic Mice

Transgenic mice expressing full-length huntingtin may provide better insights into the events and factors determining the selective cell vulnerability typical of HD, since the

Table 1
Animal Models of Huntington's Disease

Genetic model	Promoter and number of CAG	Genetic background	Protein expression level	Behavioral phenotype	Neuropathology	Inclusions	Refs.
HD exon 1	HD promoter. 144Q (expanded to 170–190Q),	CBA/C57B16	RNA levels <1X	Onset at 2 mo. Tremor and abnormal gait, learning deficit, hypokinesis, diabetes	Overall brain atrophy. Cell loss in frontal cortex, dorsal striatum, and Purkinje cells at late stage.	NII and neuropil aggregates throughout brain, fewer dendritic spines.	*81*
HD N171	PrP promoter. 82Q, 44Q, 18Q	C3H/B16	1/5X to 1/10X	82Q onset at 5 mo. Tremor and abnormal gait, hypokinesis, weight loss, early death	Overall brain atrophy. Cells w/a degenerative morphology in the lateral striatum	Inclusions in striatum, cortex, hippocampus, amygdala. Diffuse nuclear accumulation of Htt protein	*89*
Inducible HD exon 1	Tet-off (CamkIIα-tTA) TetO exon 1 94Q	CBA/C57B16	>Endogenous	94Q onset in 50% by 2, 5 mo. Late onset tremor and abnormal gait.	Brain atrophy and progressive striatal atrophy	Inclusion in striatum, septum, cortex, hippocampus. Gliosis.	*90*
Complete HD gene	HD YAC Full-length 72Q, 46Q, 18Q	FVB/N	2X	72Q onset by 3 mo. Hyperactive and circling	Cell loss in striatum	Inclusions in striatum	*75*
HD Full-length cDNA	CMV promoter. 89Q;48Q, 18Q	FVB/N	5X	89Q and 48Q onset by 4 mo. Circling, hyperactivity, end-stage hypoactivity and urinary incontinence	20% cell loss in striatum of some animals	Fewer inclusions throughout the brain.	*96*

(continues)

Table 1 (continued)

Genetic model	Promoter and number of CAG	Genetic background	Protein expression level	Behavioral phenotype	Neuropathology	Inclusions	Refs.
Hdh knock-in	*Hdh* promoter. 111Q, 92Q, 50Q	129/CD1	Endogenous	ND	ND	CAG and age dependent. Htt nuclear relocalization at 1, 5 mo. Inclusion >6 mo.	*99*
	Neo and *Hdh* promoter. 111Q, 20Q	SW	<50% of endogenous	$Hdh^{nQ20/nQ111}$ have onset ~2 mo and show disorders similar to exon1-Q144 mice.	ND	Age dependent Htt nuclear relocalization and inclusion	*78*
	Hdh promoter. 80Q, 72Q	FVB-N/B16	2X	Early-onset aggressive behavior	LTP-impaired	Late inclusion, repeat instability in striatum.	*100*
	Hdh promoter. 94Q, 71Q	C57B16	~2X	ND	NMDA sensitivity. Smaller striatal cells	No inclusions.	*76*
	Hdh promoter. 150Q, 80Q	C57B1/6J	Endogenous	150Q homo. onset by 6 mo, hetero, by 15 mo. Motor task deficits, abnormal gait, hypoactivity.	Reactive gliosis	150Q NII mainly in striatum. Htt nuclear relocalization	*102*
Targeted Non-Hdh polyQ	*Hprt* locus. 146Q insert	129/C57B16	Endogenous	Onset at ~5 mo. Handling–induced seizures and early death.	ND	Inclusions throughout the brain.	*94*

Modified with permission from *http://www.hdfoundation.org*, Hereditary Disease Foundation. NII, intranuclear inclusions; mo, month; PrP, Prion protein, ND, not determined; homo., homozygotes; hetero., heterozygotes.

expanded polyQ stretch is expressed in the context of the entire huntingtin protein.

A transgenic model, expressing full-length mutant huntingtin cDNA under the control of a heterologous cytomegalovirus promoter, has been developed by Reddy et al. *(96).* In these mice, huntingtin expression is widespread in the brain and peripheral tissues. A progressive neurological phenotype is observed, beginning at 2 mo of age. Parallel with the appearance of motor symptoms, neurodegeneration and gliosis occur in striatum and cortex, and, to a lesser extent, in the hippocampus and thalamus. Apoptosis also occurs in areas typically affected in HD, supporting the hypothesis that selective neuronal vulnerability is determined by the context of the full-length protein.

The entire HD gene cloned into yeast artificial chromosomes (YACs) has also been successfully used to generate a transgenic mice model, in which the mutant HD gene is subjected to the same kind of regulation as the endogenous gene *(75).* Indeed, in this mouse model, the pattern of huntingtin expression and neurodegeneration closely resembles the human disease. Selective processing of huntingtin was also observed in the YAC mice, in which fragments of huntingtin are found in the nucleus of medium spiny neurons only. Progressive electrophysiological dysfunction, including abolishment of long-term potentiation and abnormal NMDA receptor activity, occurs early in the pathogenesis and prior to nuclear localization of huntingtin.

In all the transgenic models expressing the full-length protein, levels of mutant huntingtin expression are higher than in R6/2 mice, although motor symptoms develop much later. As discussed in the section "Huntingtin and Apoptosis", N-terminal fragments of the mutant protein must probably be generated to exert toxicity. Under this perspective, the exon-1 transgenic mice may represent a later stage of the disease, overcoming the need for the generation of toxic fragments. If, as has been suggested *(97),* a mechanism of selective vulnerability resides in the cell-specific cleavage of the mutant protein, this would explain why R6/2 mice show a more diffuse and severe phenotype and earlier disease onset.

Knock-In Models

A further improvement in the development of genetic models of HD is represented by the precise insertion of an expanded CAG repeat into the endogenous mouse huntingtin gene. This molecular strategy circumvents potential problems generated by the use of heterologous promoters to express transgenic huntingtin, by position effects operating at the integration site, or by overexpression of the transgene *(98).*

No marked neuropathological phenotype has been reported for 4/5 knock-in models developed so far *(76,78,99,100).* In these, minor abnormalities have been observed, compared to wild-type littermates. In some of the models, mutant huntingtin clearly redistributes inside the nucleus of striatal cells. N-terminal fragments of the protein accumulate in nuclear aggregates *(97,99,101,102),* where they also recruit the wild-type protein *(101).* Another knock-in mouse *(76)* shows resting membrane potentials that are more depolarized than in control mice, and increased sensitivity to NMDA, confirming data obtained in YAC transgenic mice *(75).* A more recent knock-in mouse model, in which 150 CAG repeat has been inserted into the *Hdh* gene *(102),* is found to exhibit behavioral and neurological features resembling HD (Table 1). A gene-dosage effect has also been observed in this knock-in. Homozygotes consistently develop motor and behavioral symptoms earlier than heterozygotes *(102),* although, at this stage, it is not possible to determine whether this phenomenon results from increased levels of mutant huntingtin or, rather, from complete lack of the normal protein.

Modeling HD in Flies and Worms

Although phylogenetically very distant from mammals, flies and nematodes have often been demonstrated to be excellent models for study-

ing genetics, as well as for dissecting cellular processes and even reproducing human genetic pathologies *(103,104).* Studies on these organisms take advantage of their rapid generation time, which allows reproducing, in just a few days, pathological phenotypes that develop only in years in humans, and in months in mice.

HD along with other polyQ diseases *(103)*, has been successfully reproduced in *Drosophila*, by expressing an N-terminal fragment of the human mutant protein in the photoreceptor neurons of the fly eye *(104).* Similar to the human pathology, the resulting phenotype is a striking neuronal degeneration, with the hallmarks of apoptosis, with severity and age of onset strictly correlated with the length of the polyQ expansion *(104).* Such a model of HD has been used to screen for genetic factors counteracting the neurodegeneration induced by the expanded polyQ stretch, resulting in the identification of two chaperon proteins which are able to suppress, in the fly, mutant huntingtin toxicity *(105).*

As in the fly model, in a transgenic nematode model for mutant huntingtin, in which the N-terminal fragment of the protein is expressed in the sensory neurons, progressive neurodegeneration and deficient uptake of lipophilic vital dyes occur in a polyQ-length-dependent fashion. The toxic effect is associated with the appearance of protein aggregates, but cell death is only observed when a second toxic transgene, encoding for the dominant-negative version of OSM-10 (a protein involved in regulation of cell osmolarity in nematodes), is co-expressed in the cells *(106).*

Fly and nematode models of HD may therefore provide powerful genetic means for dissecting the mechanism of neuronal degeneration, and to screen for modifiers that could be potential targets for HD therapy.

Cellular Models of HD

Various cellular systems have been developed in the attempt to mimic the disease in a cell culture dish. Although these attempts have not yet consistently produced a sufficiently good and standard cellular system in which the activity of therapeutics or chemical libraries could be assayed via high-throughput screening, they have made important contributions to knowledge on the molecular mechanisms underlying the disease. In general, these experiments have demonstrated an increased sensitivity of cultured cells expressing mutant huntingtin to apoptotic stimuli.

Transient transfection of cells of nonneuronal origin, such as human embryonic kidney (HEK) 293 cells or 2–2 monkey kidney cells, have been extremely useful in analyzing the correlation between aggregate formation and huntingtin protein size, as well as CAG repeat length *(107–109).* Immortalized HN33 cells, derived from the hippocampus, have also been utilized to study signaling pathways, in the presence of transiently transfected mutant huntingtin *(110,111).* No deep characterization is available on these cells, but these studies have indicated that the jun N-terminal kinase (JNK) pathway is activated by mutant huntingtin.

Clonal mouse striatal cells (X57), derived by somatic cell fusion of embryonic d 18 mouse striatal cells, were utilized to demonstrate the lack of correlation between neuronal survival and intranuclear inclusions *(112).* The parental cells are known to differentiate into neurofilament-positive cells, and to express an array of D1, D2, and D5 receptor mRNA, as well as DARPP32 mRNA. Functional D1-receptor binding was described, and choline acetyltransferase activity was reported in the cells *(113).* Primary CNS neurons *(114–116)* and ES cells from knock-out mice *(63,74,77)* have also been utilized with success, to manipulate huntingtin levels.

However, cellular models based on transient transfections are not ideal for long-term analyses of the events occurring in the presence of mutant huntingtin. Instead, cells that express neuronal properties and that sustain long-term expression of the transgene may be preferable. With this goal in mind, a number

of neuronal-like cells, which stably express huntingtin, have been obtained (Table 2). These can be divided into two main groups: those that overexpress exogenous huntingtin, and those that express the mutant protein at normal levels.

In a first model, pheochromocytoma 12 (PC12) cells have been utilized *(117).* The advantage of PC12 cells is that they have been exploited in various experimental paradigms, and a large number of molecular and biological details on their properties are available *(118).* In addition, PC12 cells differentiate into neuronal-like cells in the presence of nerve growth factor. Cellular defects and altered gene expression have been observed in PC12 cells expressing an N-terminal portion of mutant huntingtin *(117).* In particular, mutant huntingtin was found to localize diffusely into the nucleus, suggestive of an influence on gene transcription. Indeed, differential display polymerase chain reaction analyses, performed in the same cells, showed altered gene expression. Engineered PC12 cells expressing the N-terminal portion of mutant huntingtin also displayed abnormal morphology and lack of normal neurite development, and were more susceptible to apoptotic stimulation *(117).* Despite the relevance of such data, generation of stable subclones from parental PC12 cells may present problems. Selection of PC12 subclones, indeed, often generates multiple phenotypes, imposing the screening of a large number of colonies to verify the results.

Despite their origin from the peripheral nervous system, neuroblastoma cells have also been widely utilized for studies related to CNS functions, because they are able to differentiate into neuronal-like cells. The mouse–rat neuroblastoma–glioma hybrid cell line, NG108-15, has been stably transfected with the tetracycline-inducible transactivator and mutant huntingtin cDNA *(119).* This system allows following longitudinally the evolution of the phenotype, including the processing of the normal and mutant huntingtin, as well as aggregate formation and localization with time.

Although inducible systems should be pursued, neuroblastoma cells are, like PC12 cells, of tumoral origin, and the genetic events that lead to their continous growth are unknown. In this respect, a more reliable system is represented by conditionally immortalized cells, which also offer the possibility of being derived from the brain region of choice. ST14A cells were obtained, more than 10 yr ago, from the embryonic striatum via immortalization with a temperature-sensitive variant of the large-T antigen *(120).* These cells are easy to genetically engineer, and can generate subclones that stably maintain the properties of the original line *(121).* ST14A cells divide in vitro at 33°C, but stop dividing at 39°C and, in vivo, after intracerebral transplantation *(120,122–124).* Exposure to 39°C guarantees inhibition of cell proliferation, but differentiation in these conditions is obtained with difficulty. Indeed, ST14A cells exposed to serum-deprived medium at 39°C tend to change morphology, then die progressively by apoptosis *(125).* Cells death is postponed, if cells are cultured in complete medium. However, exposure of ST14A cells to a differentiating mix produces morphological and electrophysiogical differentiation in a percentage of the cells *(126).* More recently, ST14A cells have been shown to express antigenic and biochemical properties found in medium-size striatal neurons. These include γ-aminobutyric acid and DARPP-32 expression, D1- and D2-like receptor activities *(126),* as well as a functional adenosine 2a receptor (A_{2a}) *(127).*

Expression of mutant huntingtin in ST14A cells accelerates apoptotic cell death in serum-deprived medium, and makes them more sensitive to proapoptotic stimuli *(125).* A synergistic interaction between mutant huntingtin and caspase-3 and Bad was also observed. Similarly, caspase-3 activity was increased in ST14A cells expressing mutant huntingtin. This evidence confirms prior observations of increased apoptotic cell death in lymphocytes obtained from HD patients

Table 2
Modeling Huntington's Disease in Neuronal Cells

Htt-stable neuronal cell lines	cDNA(s)/ Method of delivery	Resulting expression	Phenotype analyzed (death/other)	Aggregates	Ubiquitin	Refs.
Neuroblastoma NG108-15	FL(15Q) FL(73Q) FL(116Q) N502(15Q) N502(73Q) N502(116Q) N80(15Q) N80(73Q) N80(116Q) /transfection	Stable Htt inducibles (TetOn)	Apoptotic cell death in 30% of the Flmu cells after 16 d in differentiating mix. Earlier death in N508 and N73mu cells	CI and NI in muHtt cell is time- and polyQ-dependent Rapid inclusion formation in N508 and N73. Inclusions correlate with apoptosis. Processing of FLmuHtt	Inclusions are ubiquitinated	*119*
PC12[118]	N67(20Q) N67(150Q) /transfection	Stable overexpressants	Spontaneous apoptotic cell death in N67(150Q) muHtt cells are sensitive to staurosporine impaired response to NGF Nuclear toxicity altered gene expression Increased expression of Caspase-1	Intranuclear localization of N67(150Q)	ND	*117*
ST14A[120–124,126]	FL(20Q) FL(82Q) N548(15Q) N548(120Q) N63(18Q) N63(82Q) /transfection /retroviral transduction	Stable overexpressants	Apoptotic cell death after SDM, 3NP, and death genes More death in N548 and N63 mu cells Leakage of cytochromec in muHtt cells (Cattaneo, unpublished) Impaired A2A signaling wtHtt is antiapoptotic	ND	ND	*125* *127*
STHdhQ111	Conditional immortalization from knock; in mice[99]	Normal expression of mutant Htt	Sensitivity to hypoxia Elevated p53 levels Abnormal ER response Heightened basal activity of the iron pathway	Alternative forms of Htt in the nucleus and cytoplasm	ND	*130*

FL, full length; N, N-terminal; Q, glutamine; ER, endoplasmic reticulum; CI, cytoplasmic inclusions; NI, nuclear inclusions; SDM, serum deprived medium, ND, not determined.

(128), indicating that similar mechanisms occur in human cells and in a cell culture dish. A similar experiment, performed in PC12 cells, gave the same result *(111),* providing additional evidence in favor of an overactivation of caspase-3. More recently, engineered ST14A cells have been exploited to evaluate whether mutant huntingtin interferes with signaling from the A_{2a}/D2 striatal receptors. Aberrant amplification of A_{2a} signaling occurs in ST14A cells expressing mutant huntingtin, and this effect can be fully counteracted by selective A_{2a} antagonists *(127).*

Given the propensity of ST14A cells to undergo apoptotic cell death in serum-deprived medium, this paradigm was chosen to test whether normal huntingtin could interfere with cell apoptosis. Rigamonti et al. *(125)* found that ST14A cells overexpressing normal huntingtin are protected from death induced by serum deprivation, 3-nitropropionic acid exposure, or transfection of death genes. These results have recently been confirmed in vivo, and lead to the proposition that loss of huntingtin neuroprotective activity may contribute to HD *(34).*

A further advance in the production of huntingtin-engineered cells is based on the conditional immortalization of striatal cells obtained from transgenic HD animals. In particular, neuronal progenitor cell lines were established from E14 striatal primordia of Hdh^{Q111} knock-in and wild-type littermate embryos *(129),* by virtue of the same strategy utilized to generate ST14A cells. Multiple clonal lines of nestin-positive cells from the wild-type and the heterozygous mutant embryos were established *(130).* In contrast, only two clones from the mutant homozygote embryos were successfully passaged. This represents the only cell model in which the mutant protein is expressed at physiological levels.

Although something is learned from all these cellular models, progress also urgently needs to be made toward the production of a neuronal cell system that more reproducibly recapitulates aspects of the disease, including massive cell death coinciding with mutant huntingtin expression.

Lessons from Models

Mutant Huntingtin Aggregates: Cause or Effect?

A striking feature, shared by mutant huntingtin and other proteins involved in CAG-repeat diseases, is their tendency to form insoluble aggregates *(131),* either through "polar zippers" *(132)* or by transglutaminase-mediated events *(133).*

Ubiquitinated aggregates of N-terminal mutant huntingtin accumulate in striatal and cortical neurons of HD patients *(134,135),* both in the nucleus and dystrophic neurites *(136).* Huntingtin aggregates are also common to all transgenic models developed, although description of their nuclear localization is reported to vary from 0.1 *(137)* to 100% *(81)* of the striatal neurons, depending on the mouse model.

Cell culture studies showed that a longer polyQ string (and a shorter protein fragment in which it is contained) correlates with a greater frequency of aggregate formation *(107–109).*

Whether aggregates in HD can be causative of the pathology, or, rather, whether they represent an epiphenomenon *(138,139),* is still debated. The latter hypothesis is substantiated by cell studies showing that neurodegeneration and cell death do not correlate with mutant huntingtin aggregation *(114,112),* but, rather, to nuclear localization of the mutant protein *(114).* Similarly, a soluble form of mutant huntingtin is found to affect synaptic activity in the absence of aggregates *(97),* and neurodegeneration occurs in the absence of aggregates in the YAC HD model *(75).*

More recent studies have demonstrated that the suppression of aggregate formation by overexpression of heat shock proteins results in reduced polyQ-induced toxicity/cell death *(140–142).* However, the effect of heat shock

proteins may vary, depending on the cell lines used *(143),* and may also be related to their intrinsic property of counteracting apoptosis *(144).* Similarly, peptides that bind the extended polyQ domain are able to inhibit aggregation, both in vitro and in cells, and to reduce cell death *(145).* Mutant huntingtin toxicity has not yet been conclusively proven to be mediated by its aggregation, and a clear understanding of what mutant huntingtin inclusions do inside the cells is crucial. Indeed, therapies aimed at reducing aggregate formation could result in worsening the general scenario of the pathology, in the case that soluble N-terminal fragments of the mutant protein are the real toxic species, and aggregates are only the means to sequestrate them into an inactive form *(146).*

Huntingtin Interacting Proteins: Looking for Hints

The toxicity of mutant huntingtin may derive from abnormal interactions with other proteins, as a consequence of conformational changes induced by the expanded polyQ stretch. The search for a huntingtin partner, which would explain the toxicity of the expanded CAG and the selective vulnerability in HD, is still active. So far, several proteins have been identified as potential partners of huntingtin, but no interacting protein by itself can explain the pathology. When looking at huntingtin interactors (Table 2), evidently most of them can be grouped into three main categories that may give clues to huntingtin function. One category is represented by proteins involved in membrane trafficking, clathrin-mediated endocytosis, and recycling of synaptic vesicles *(147–150).* All of them are able to interact with both wild-type and mutant huntingtin, but the length of the polyQ modulates the strength of the interaction.

Huntingtin-interacting protein 1 (HIP-1) *(148,149)* is a homolog of Sla2p, a yeast protein involved in actin organization and endocytosis, and it has also been demonstrated to function as a proapoptotic protein containing a novel caspase-dependent death effector domain *(151).* HIP-1 might be a molecular accomplice in the pathogenesis of HD. Indeed, the interaction of HIP-1 with mutant huntingtin is weaker than with the wild-type protein *(148,149),* and it has been suggested that the inability of mutant huntingtin to efficiently sequester HIP-1 may result in an increased rate of apoptosis *(151).*

Another interacting protein involved in membrane trafficking is huntingtin-associated protein 1 (HAP-1) *(147).* A recent study has shown that this protein is concentrated in axon terminals, and is associated with synaptic vesicles, in which it seems to have a role in promoting neurite extension *(152).* When this protein is co-transfected with mutant huntingtin in PC12 cells, it becomes recruited into huntingtin aggregates, and it is no longer able to stimulate neurite growth *(152).* Although data on HAP-1 are suggestive of a role of mutant huntingtin in altering synaptic functions (an hypothesis also substantiated by data obtained in transgenic models *[97]*), it is not yet clear whether an aberrant interaction with HAP-1 may actually contribute to HD. In fact, a domain included between amino acids 171 and 230 of mutant huntingtin seems to be essential for interaction with HAP-1 *(153),* but, in a transgenic exon-1 model of HD *(81),* in which the domain responsible for the interaction is not present, neuropathology and sympthomatology still mirror HD.

A second group of proteins interacting with huntingtin includes those involved in transcriptional events and transcriptional regulation (154–156; Table 3). In all these proteins, the region of huntingtin involved in the interaction is the same, i.e., the poly-Pro stretch, but the length of the CAG expansion modulates the strength of interaction. This is not completely unexpected, since poly-Pro stretches are often involved in protein–protein interactions *(157,158),* but it also raises the possibility that some of the interactions found may not be specific.

Interactors of the third group are involved in signaling *(111,159,160;* Table 3). Specifically,

Table 3
Huntingtin's Interacting Proteins

Interacting protein	Method of identification	Dependence of interaction from CAG length	Region of huntingtin involved	Function of protein	Ref.
Proteins involved in membrane trafficking					
HAP-1	Yeast two-hybrid system	Stronger interaction with increasing CAG lenght	N-terminal (aa. 1–230) aa. 171–230 are essential for the interaction	Involved in membrane trafficking	*147*
HIP-1	Yeast two-hybrid system	Weaker interaction with increasing CAG length	N-terminal (aa. 9–294)	Pro-apoptotic, homolog to Sla2p in yeast, protein involved in actin organization and endocytosis	*148,149*
SH3GL3	Co-immunoprecipitation and co-localization	Stronger with mutant Htt	Poly-Pro region	Clathrin-mediated endocytosis and recycling of synaptic vescicles	*150*
Proteins involved in transcriptional events					
HYP-A, -B, -C	Yeast two-hybrid system	Stronger interaction with increasing CAG length	Poly-Pro region	WW-domain proteins. HYP-A/FBP-11 and HYP-C are involved in mRNA splicing; HYP-B is a transcription factor	*154*
N-CoR	Yeast two-hybrid system	Stronger interaction with increasing CAG length	N-terminal (aa. 1–171)	Nuclear receptor co-repressor	*155*
CREB-binding protein and mSin3a	GST pull-down	Weak interaction little increase with increasing CAG length	Poly-Pro region	Transcriptional coactivator and corepressor, respectively	*156*
p53	GST pull-down	No	Poly-Pro region	Transcription factor	*156*

(continues)

Table 3 (continued)

Interacting protein	Method of identification	Dependence of interaction from CAG length	Region of huntingtin involved	Function of protein	Ref.
Proteins involved in signalling events					
MLK2	Co-immunoprecipitation and GST pull-down	Weaker interaction with increasing CAG length	First three exons	JNK activator	*111*
Grb2 and RasGAP	Co-immunoprecipitation	Not known	Not known	Signaling proteins	*159*
She and EGF receptor	Co-immunoprecipitation	Not known	Indirect interaction	Signaling proteins	*159*
Calmodulin	Co-elution by gel filtration	Stronger with mutant Htt	Indirect interaction, Ca^{2+}-dependent	Ca^{2+} binding regulatory protein	*160*
Proteins with other functions					
Cystathionine B-synthase	Yeast two-hybrid system	No	N-terminal (aa. 1–171)	Metabolic enzyme	*161*
GAPDH	Affinity chromatography	Stronger interaction with increasing CAG length	Poly-Gln stretch	Glycolitic enzyme, translational regulator	*162*
HIP-2	Yeast two-hybrid system	No	N-terminal (aa. 1–540)	Ubiquitin-conjugating enzyme	*163*
HAP-40	Co-immunoprecipitation	Not known	C-terminal	not known	*164*

CREB, Cyclic AMP response element binding factor; EGF, epidermal growth factor; FBP-11, formin-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Grb2, growth factor receptor bound protein 2; GST, glutathione S-transferase; HAP-1, -40, huntingtin associated protein -1, -40; HIP-1, -2, huntingtin interacting protein -1, -2; HYP-A, -B, -C, huntingtin yeast partners -A, -B, -C; MLK2, mixed-lineage kinase 2; N-CoR, nuclear receptor corepressor; RasGAP, Ras GTPase-activating protein; JNK, jun N-terminal kinase; Shc, Src-homolog and collagen homolog; SH3GL3, SH3-containing Grb2-like protein.

huntingtin was found to be directly associated with the SH3 domains of Grb2 and Ras GAPase activating protein, upon activation of epidermal growth factor receptor *(159),* suggesting that huntingtin may be involved in the regulation of Ras-dependent signaling pathways. Interaction with the mixed-lineage kinase 2 (MLK2), a kinase involved in the mitogen-activated protein kinase cascade, has been observed, as well *(111).*

Finally, a few proteins interacting with huntingtin do not fit into any of the three categories mentioned above *(161–164).* Glyceraldeyde-P-dehydrogenase (GAPDH) is one of them and its aberrant interaction with huntingtin argues in favor of metabolic and energy impairment in HD *(165).* However, this interaction is not specific because GAPDH also interacts with the dentatorubral-pallidoluysian atrophy (DRPLA) protein *(162).*

Recently, a 40-kDa protein, HAP-40, has been isolated, which co-immunoprecipitates with full-length huntingtin, but not with its N-terminal fragment. HAP-40, whose function is still unknown, is distributed in the cytoplasm, in the presence of huntingtin, but becomes actively targeted to the nucleus in its absence *(164).* The implication of this interaction, in terms of providing clues on normal huntingtin function or mutant huntingtin toxicity, remains to be investigated.

If all the identified huntingtin interactors really interact with huntingtin in vivo, one should imagine huntingtin as a sort of molecular glue. Indeed, mutant huntingtin aggregates do recruit several proteins *(166),* most of which are transcription factors and regulators. Alternatively, interactions with different proteins might be subjected to some kind of regulation.

Huntingtin Functions and Mutant Huntingtin Dysfunction

Various lines of evidence suggest that normal huntingtin function and mutant huntingtin toxicity are faces of the same coin, i.e., that HD may be caused by the gain-of-function toxicity of the expanded CAG, and by the loss of normal huntingtin function *(34).* Under this new perspective, searching for normal huntingtin function might lead to a better understanding of the neuropathology and new therapeutical strategies. Despite the early recognition of the essential role of huntingtin for embryonic development *(69–71)*, only recently has its function in the adult begun to be elucidated.

Huntingtin and Intracellular Vesicle Trafficking

Diverse evidence converges to indicate that normal huntingtin might be involved in cellular vesicle trafficking and endocytosis. As already mentioned, several proteins interacting with huntingtin are associated with membrane vesicles and interact with cytoskeletal proteins (Table 3). Huntingtin also co-distributes with intracellular and plasma membranes containing clathrin *(167),* and is recruited with clathrin to plasma membranes, following stimulation of adenylyl cyclase or dopamine D1 receptor *(168).*

Another set of investigations has speculated that huntingtin may have a role in retrograde and fast axonal transport *(169).* Redistribution of huntingtin from the cell body to dendrites, neurites, and synaptic boutons, upon stimulation of adenylyl cyclase or dopamine D1 receptor, has been demonstrated *(168).* In addition, synaptic vesicles incubated with N-terminal fragments of mutant huntingtin show impaired glutamate uptake in vitro *(97).* Data obtained in HD brains and mice *(75,97,134),* and in the conditional huntingtin knockout mice *(79),* also show degeneration of axon fibers, a finding that is compatible with the above hypothesis of a function of huntingtin in cellular trafficking and synaptic vesicles.

In knockout striatal cells abnormal distribution of nucleoli, nuclear transcription factor-speckles, Golgi apparatus, mitochondria and endoplasmic reticulum has been demonstrated *(63),* suggesting that huntingtin may be implicated in perinuclear processes essential for normal trafficking of secretory membranes,

and for the assembly of mitochondria near the nucleus. It has also been suggested that the toxicity of polyQ stretches might be determined by their ability to form nonselective ion channels in phospholipids membranes *(170).* This property is enhanced by acidic pH, making mitochondrial and endosome/lysosomes preferential targets for polyQ action. Indeed, a potential action of mutant huntingtin on the endosomal/lysosomal system has been demonstrated *(171).* In aberrant conditions, a process that is normally finely regulated *(168)* may turn destructive, via activation of the endosomal–lysosomal system, and subsequent autophagy *(171).* How cell death occurs in HD is still under intense investigation, and it is not clear whether autophagy may be of relevance.

Huntingtin and Transcriptional Regulation

Huntingtin protein sequence does not predict its physiological role, but a striking feature in the structure of huntingtin is the presence of a long polyQ stretch followed by a polyproline domain. These structures are primarily associated with transcriptional regulatory proteins *(172),* and their length influences the transcriptional activity of the protein. Glutamine stretches longer than 40 show a reduced ability to mediate transcriptional activation *(172).* Although huntingtin does not seem to have a DNA-binding domain, its N-terminal structure is suggestive of an action as a modulator of transcriptional events through interaction with transcription factors *(166).* Several studies have recently supported this hypothesis. Huntingtin specifically binds the nuclear receptor co-repressor, in a CAG length-dependent fashion *(155).* Upon binding to normal huntingtin, this protein remains mostly in the cytoplasm, while in cortical and striatal neurons of HD brains it redistributes into the nucleus *(155).* Furthermore, in a cell model, exon-1 huntingtin interacts with p53 and, when the CAG is extended, it represses transcription of p53-regulated promoters *(156).* These data suggest that huntingtin normally interacts with transcriptional regulatory proteins, and has a role in their transport within the cytoplasm and inside the nucleus. The length of the polyQ region may affect the kinetics of association between huntingtin and the transcriptional proteins, thus altering their intracellular distribution and availability *(155).*

Huntingtin also interacts with a family of WW domain proteins closely related to nuclear-binding proteins *(173)* and co-localizes with specific nuclear structures *(130).* On the basis of these observations, Trettel et al. *(130)* has suggested that huntingtin may be implicated in the transport and processing of mRNA. A possible role of huntingtin in cytoplasm–nucleus shuttling is also suggested by its only known structural domains, i.e., HEAT domains *(174),* which have also been found in nuclear shuttle proteins, such as the importins *(175).* In addition, translocation of huntingtin or its N-terminal fragment to the nucleus might be directed by a nuclear localization signal, potentially present in the N-terminus of the protein *(176).*

Huntingtin and Apoptosis

Studies conducted by the authors have provided the first direct evidence for a role of huntingtin on the survival of CNS cells *(125).* Striatal cells engineered to express wild-type huntingtin were indeed resistant to the lethal effect of stresses, such as serum deprivation, exposure to 3-nitropropionic acid or transfection of death genes. In particular, huntingtin was found to have an antiapoptotic action that is exerted downstream of proapoptotic Bcl-2 members and upstream of caspase-3 activation *(125).* This evidence argue in favor of a loss-of-function mechanism contributing to HD *(34).*

In the presence of mutant huntingtin, cells were more susceptible to induction of apoptosis by toxic stimuli and, indeed, caspase-3 was more active than in controls *(125).* In addition, a leakage of cytochrome c was found in those cells, even in the absence of apoptotic stress (Cattaneo et al., unpublished). Leakage of cytochrome C, activation of caspase-3 and, in addition, increased

expression of caspase-1 in the presence of an N-terminal fragment of mutant huntingtin, is also reported by Li et al. *(177).*

A recent report indicates that huntingtin antiapoptotic effect occurs via sequestration of proapoptotic HIP-1 *(151).* Given the specific distribution of HIP1 in the brain (although expression is not restricted to striatum), Hackam et al. *(151)* suggested that the inability of mutant huntingtin to modulate HIP-1 toxicity contributes to the amplification cascade of cell-death signals in HD through the same pathway identified by Rigamonti et al. *(125).*

Caspase-1-activated pathways appear to be common to several other neuropathologies, including Parkinson's disease *(178),* amyotrophic lateral sclerosis *(179)* and ischemia *(180),* and they appear to play an important role in HD pathogenesis, as well *(51).* Caspase-1 activity has been found to be higher in the brains of HD patients and R6/2 mice *(51).* When these mice were crossbred with a transgenic mouse expressing a dominant-negative mutant of caspase-1, double transgenics showed a delayed onset and slower progression of the disease *(51).* The same result was achieved via inhibition of caspase-1 and caspase-3 expression by minocycline *(181).*

Huntingtin is itself a substrate for caspases -1, -3, and -6 *(182,183),* and abrogation of its cleavage lessens the toxicity of the mutant protein in cell models *(184).* Caspase-1-dependent cleavage of endogenous wild-type huntingtin has been shown in R6/2 mice *(51),* along with a reduced expression of the endogenous protein in hippocampus, striatum, cortex and cerebellum. In mice lacking caspase-1, together with a general amelioration of the pathology, normal levels of endogenous huntingtin were restored *(51).* Also, this observation argues in favor of a protective function of wild-type huntingtin. Yet, although data are accumulating suggesting an involvement of caspase-1 in HD pathology, previous studies on fibroblasts *(116)* had shown that caspase-1, -2, -3, and -11 did not play a critical role in polyQ-induced cell death. Discrepancies among the studies could result from differences in the cell models used, and, possibly, from the fact that, in the latter study, the polyQ stretch by itself was used.

Caspase-8 is another potential target of polyQ repeats. Its critical role in polyQ repeat-induced cell death has been shown in cell lines and rat primary neurons *(116,185).* In an inducible cell model for expanded CAG, activation of caspase-8 was found to precede activation of caspase-3 *(185).* Also, activated caspase-8 has been found in caudate, but not in cerebellum, of HD and control brains *(116).*

Other death pathways are activated in the presence of expanded polyQ repeats. Activation of JNK and neuronal apoptosis were observed in a hippocampal-derived cell line expressing mutant huntingtin *(110).* Wild-type huntingtin normally interacts with and inhibits the MLK2 *(111),* a kinase that indirectly activates JNK *(186,187).* Mutant huntingtin, instead, binds MLK2 less efficiently, suggesting that the highest concentration of free (thereby active) MLK2, in cells expressing mutant huntingtin, may lead to JNK-mediated apoptosis *(111).* Indeed, co-expression of a dominant-negative form of MLK2 with full-length mutant huntingtin dramatically reduces neuronal toxicity mediated by mutant huntingtin. This may be another example in which loss of wild-type huntingtin function is responsible for at least part of the toxic effects induced by the mutant protein. This is also corroborated by the fact that co-expression of an N-terminal fragment of wild-type huntingtin rescues cells from toxicity induced by MLK2 and mutant huntingtin *(111).* In agreement with the observations of huntingtin beneficial functions, increasing the expression of wild-type huntingtin in transgenic mice protects against the toxic effects of mutant huntingtin *(80).* YAC72 mice lacking endogenous wild-type huntingtin, exhibited significant cell death at an early age, in contrast to minimal cell death in YAC72 mice with two copies of the normal endogenous allele, and intermediate cell death in YAC72 heterozygous mice *(80).*

According to their central role in apoptosis, mitochondria dysfunction has been also

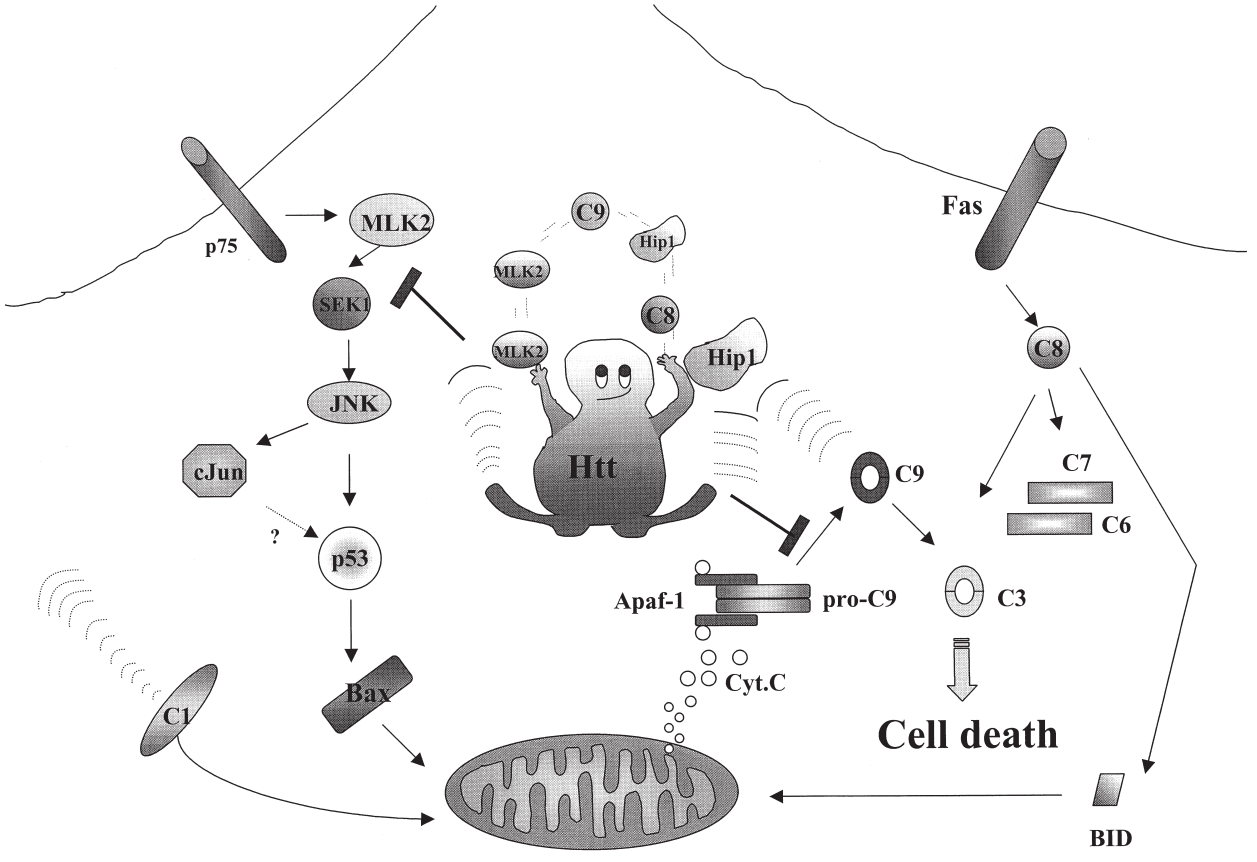

Fig. 1. Huntingtin, the key player. Schematic drawing summarizing some of the interactions of huntingtin with key cellular elements involved in apoptotic cascades. Htt, huntingtin; MLK2, mixed-lineage Kinase 2; JNK, jun N-terminal kinase; C9, caspase-9; C8, caspase-8; C7, caspase-7; C6, caspase-6; pro-C9, pro-caspase-9; CytoC, cytochrome c.

reported in HD brains *(188)* and in lymphoblasts *(128)*, probably associated with a defect in Ca^{2+} handling *(189)*.

Different pathways of cell toxicity and death may be activated by mutant huntingtin. Each of these pathways may have major relevance, depending on the cell type and context in which they are evaluated. Perhaps huntingtin is central among different death pathways (Fig. 1). Loss of its normal function, from one side, and stress-responses activated by the expanded polyQ stretch, on the other side, may act synergistically, first determining a state of increased susceptibility to stress stimuli and, eventually, cell death. A lethal feed-forward cycle is also postulated, in which sub-lethal stresses activate caspases which in turn, cleave expanded polyQ-containing proteins. The toxic fragments released would further activate caspases, amplifying stress responses and leading to cell death *(185)*.

According to this hypothesis, it has recently been demonstrated that the neuropathological decline in HD patients can be described by a mathematical model in which the probability of neuronal death remains constant with age *(190)*. In other words, the time of death of an individual neuron would be randomly determined. On the basis of this mathematical analysis, it has been proposed that neurons

bearing the mutation would be in an abnormal homeostatic state, only slightly different from the normal neuronal steady state, yet associated with an increased risk of cell death. Different mutations (i.e., different lengths of CAG expansion) would shift, to various extents, the steady state of neurons closer to the cell-death threshold, thereby increasing, for neurons, the chance of exceeding that threshold and dying *(190).* This model would argue against HD being a late-onset disease, but rather suggests that the disease is constantly active throughout a life-span.

Selective Vulnerability

The specific vulnerability of striatal neurons in HD remains unexplained. Neither Huntingtin interactors, nor their expression and distribution in the brain, neither its effects on apoptotic pathways, may be taken into account to understand the cell specificity of the neurodegeneration in HD *(34).* Alternatively, striatum-specific transcriptional regulatory proteins could be more vulnerable to huntingtin dysfunction, and lead to a progressive transcriptional dysregulation, but this remains, so far, a mere speculation.

Recently, Li et al. *(97)* has suggested that striatal vulnerability in HD may rely on a striatum-specific processing of the full-length protein. Two different studies have demonstrated a selective accumulation of N-terminal fragments in striatal neurons and their axonal projections in knock-in *(97)* and full-length HD mice *(75).*

Finally, the selective vulnerability may depend on dysfunction in brain areas that normally project to the striatum. Because huntingtin is highly localized in all corticostriatal neurons *(49),* a defect in huntingtin function, or the acquired activity of mutant huntingtin, may render corticostriatal neurons destructive, rather than rendering striatal neurons vulnerable. Evidence that degeneration and/or dysfunction of corticostriatal projections occur at early stages of the disease also come from HD patients, in which accumulation of mutant huntingtin or its N-terminal fragments occurs in corticostriatal nuclei, cytoplasm, and dystrophic neurites *(191).*

Conclusions

This review gives an overview on current issues in HD research. Most discoveries about this disease have been made thanks to animal and cell models. Pathways of cell death, identified in these models, have disclosed new perspectives on the cure of the disease by potential inhibitors of death effectors. The recognition of an important neuroprotective function of normal huntingtin has led to rethinking on the impact of a gene-therapy approach aimed at restoring huntingtin levels, or at abolishing the production of the mutant protein. The discovery that neurodegeneration, induced by mutant huntingtin, may be blocked and, at least for some aspects, reversed, has provided new hope for HD patients at the beginning of their affliction. At the same time, although HD has been traditionally considered a late-onset disease, the finding that neuronal dysfunction starts years before clinical symptoms appear has suggested the importance of developing drugs useful for counteracting the disease at its real appearance (which could actually be at the beginning of a patient's life). The puzzle is still far from being complete, but researchers will eventually find and fit together all the pieces.

Note added in proof: While this paper was in press, we demonstrated that striatal vulnerability is likely linked to the loss of BDNF-producing activity of normal huntingtin (Zuccato et al., 2001, *192*). Also, Nucifora et al. (2001, *193*) have recently demonstrated that huntingtin binds to CREB-binding protein (CBP), impairing gene transcription. It becomes more and more evident that HD is a dual disease, from one side the toxic gained activity of mutant huntingtin, from the other, the loss of the beneficial activity of normal huntingtin.

Acknowledgments

The authors thank Valenza Marta and Dr. Alessandra Foietta for their help in the preparation of the manuscript. The work of the authors described in this review is supported by the Huntington's Disease Society of America (HDSA), the Hereditary Disease Foundation (HDF), Telethon (E840) and Ministero dell'Universita' e della Ricerca Scientifica (MURST MM06278849-005) to Elena Cattaneo. Elena Cattaneo is a member of the "Coalition for the Cure" (HDSA) and of the "Cure Initiative" (HDF).

References

1. Folstein S. E. (1989) *Huntington's Disease: A Disorder of Families.* Johns Hopkins University Press, Baltimore, MD, pp. 100–136.
2. Harper P. (1996) *Huntington's Disease.* W.B. Saunders, London, pp. 317–358.
3. Nance M. A. (1998) Huntington disease: clinical, genetic, and social aspects. *J. Geriatr. Psychiatry Neurol.* **11,** 61–70.
4. Brinkman R. R., Mezei M. M., Theilmann J., Almqvist E., and Hayden M. R. (1997) The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. *Am. J. Hum. Genet.* **60,** 1202–1210.
5. Smith M. A., Brandt J., and Shadmehr R. (2000) Motor disorder in Huntington's disease begins as a dysfunction in error feedback control. *Nature* **403,** 544–549.
6. Sanberg P. R., Fibiger H. C., and Mark R. F. (1981) Body weight and dietary factors in Huntington's disease patients compared with matched controls. *Med. J. Aust.* **1,** 407–409.
7. Nance M. A. and Sanders G. (1996) Characteristics of individuals with Huntington disease in long-term care. *Mov. Disord.* **11,** 542–548.
8. Pratley R. E., Salbe A. D., Ravussin E., and Caviness J. N. (2000) Higher sedentary energy expenditure in patients with Huntington's disease. *Ann. Neurol.* **47,** 64–70.
9. Stoy N. and McKay E. (2000) Weight loss in Huntington's disease. *Ann. Neurol.* **48,** 130–131.
10. Hayden M. R. (1981) *Huntington's Chorea.* Springer-Verlag, New York, pp. 140–189.
11. van Dijk J. G., van der Velde E. A., Roos R. A., and Bruyn G. W. (1986) Juvenile Huntington disease. *Hum. Genet.* **73,** 235–239.
12. MacMillan J. and Quarrell O. (1996) The neurobiology of Huntington's disease, in *Huntington's Disease* (Harper, P., ed.), W.B. Saunders, London, pp. 317–358.
13. Reiner A., Albin R. L., Anderson K. D., D'Amato C. J., Penney J. B., Young A. B. (1988) Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl. Acad. Sci. USA* **85,** 5733–5737.
14. Albin R. L., Young A. B., Penney J. B., Handelin B., Balfour R., Anderson K. D., et al. (1990) Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease. *N. Engl. J. Med.* **322,** 1293–1298.
15. Albin R. L., Reiner A., Anderson K. D., Dure L. S., 4th, Handelin B., Balfour R., et al. (1992) Preferential loss of striatoexternal pallidal projection neurons in presymptomatic Huntington's disease. *Ann. Neurol.* **31,** 425–430.
16. Sotrel A., Paskevich P. A., Kiely D. K., Bird E. D., Williams R. S., and Myers R. H. (1991) Morphometric analysis of the prefrontal cortex in Huntington's disease. *Neurology* **41,** 1117–1123.
17. Hedreen J. C., Peyser C. E., Folstein S. E., and Ross C. A. (1991) Neuronal loss in layers V and VI of cerebral cortex in Huntington's disease. *Neurosci. Lett.* **133,** 257–261.
18. Vonsattel G. J. P. and DiFiglia M., (1998) Huntington's Disease. *J. Neuropathol. Exp. Neurol.* **57,** 369–384.
19. Lodi R., Schapira A. H., Manners D., Styles P., Wood N. W., Taylor D. J., Warner T. T. (2000) Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. *Ann. Neurol.* **48,** 72–76.
20. Gusella J. F., Wexler N. S., Conneally P. M., Naylor ,S. L., Anderson M. A., Tanzi R. E., et al. (1983) A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* **306,** 234–238.
21. Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosome. *Cell* **72,** 971–983.
22. Ashizawa T., Wong L. J., Richards C. S., Caskey C. T., and Jankovic J. (1994) CAG

repeat size and clinical presentation in Huntington's disease. *Neurology* **44,** 1137–1143.
23. Gusella J. F., MacDonald M. E., Ambrose C. M., and Duyao M. P. (1993) Molecular genetics of Huntington's disease. *Arch. Neurol.* **50,** 1157–1163.
24. Brandt J., Bylsma F. W., Gross R., Stine O. C., Ranen N., and Ross C. A. (1996) Trinucleotide repeat length and clinical progression in Huntington's disease. *Neurology* **46,** 527–531.
25. Trottier Y., Biancalana V., and Mandel J. L. (1994) Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset. *J. Med. Genet.* **31,** 377–382.
26. Ranen N. G., Stine O. C., Abbott M. H., Sherr M., Codori A. M., Franz M. L., et al. (1995) Anticipation and instability of IT-15 (CAG)n repeats in parent-offspring pairs with Huntington disease. *Am. J. Hum. Genet.* **57,** 593–602.
27. Rubinsztein D. C., Leggo J., Chiano M., Dodge A., Norbury G., Rosser E., and Craufurd D. (1997) Genotypes at the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington disease. *Proc. Natl. Acad. Sci. USA* **94,** 3872–3876.
28. Vuillaume I., Vermersch P., Destee A., Petit H., and Sablonniere B. (1998) Genetic polymorphisms adjacent to the CAG repeat influence clinical features at onset in Huntington's disease. *J. Neurol. Neurosurg. Psychiatry* **64,** 758–762.
29. Rubinsztein D. C., Barton D. E., Davison B. C., and Ferguson-Smith M. A. (1993) Analysis of the huntingtin gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of onset of Huntington's disease and CAG repeat number. *Hum. Mol. Genet.* **2,** 1713–1715.
30. Andrew S. E., Goldberg Y. P., Theilmann J., Zeisler J., and Hayden M. R. (1994) A CCG repeat polymorphism adjacent to the CAG repeat in the Huntington disease gene: implications for diagnostic accuracy and predictive testing. *Hum. Mol. Genet.* **3,** 65–67.
31. Almqvist E., Spence N., Nichol K., Andrew S. E., Vesa J., Peltonen L., et al. (1995) Ancestral differences in the distribution of the delta 2642 glutamic acid polymorphism is associated with varying CAG repeat lengths on normal chromosomes: insights into the genetic evolution of Huntington disease. *Hum. Mol. Genet.* **4,** 207–214.
32. Snell R. G., MacMillan J. C., Cheadle J. P., Fenton I., Lazarou L. P., Davies P., et al. (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat. Genet.* **4,** 393–397.
33. Farrer L. A., Cupples L. A., Wiater P., Conneally P. M., Gusella J. F., and Myers R. H. (1993) The normal Huntington disease (HD) allele, or a closely linked gene, influences age at onset of HD. *Am. J. Hum. Genet.* **53,** 125–130.
34. Cattaneo E., Rigamonti D., Goffredo D., Zuccato C., Squitieri F., Sipione S. (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. *Trends Neurosci.,* **24,** 182–188.
35. Andrew S. E., Goldberg Y. P., Kremer B., Telenius H., Theilmann J., Adam S., et al. (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat. Genet.* **4,** 398–403.
36. Barron L. H., Rae A., Holloway S., Brock D. J., and Warner J. P. (1994) A single allele from the polymorphic CCG rich sequence immediately 3′ to the unstable CAG trinucleotide in the IT15 cDNA shows almost complete disequilibrium with Huntington's disease chromosomes in the Scottish population. *Hum. Mol. Genet.* **3,** 173–175.
37. Squitieri F., Andrew S. E., Goldberg Y. P., Kremer B., Spence N., Zeisler J., et al. (1994) DNA haplotype analysis of Huntington disease reveals clues to the origins and mechanisms of CAG expansion and reasons for geographic variations of prevalence. *Hum. Mol. Genet.* **3,** 2103–2114.
38. Monckton D. G. and Caskey C. T. (1995) Unstable triplet repeat diseases. *Circulation* **91,** 513–520.
39. Aronin N., Chase K., Young C., Sapp E., Schwarz C., Matta N., et al. (1995) CAG expansion affects the expression of mutant huntingtin in the Huntington's disease brain. *Neuron* **15,** 1193–1201.
40. Kennedy L. and Shelbourne P. F. (2000) Dramatic mutation instability in HD mouse striatum: does polyglutamine load contribute to cell-specific vulnerability in Huntington's disease. *Hum. Mol. Genet.* **9,** 2539–2544.
41. Mangiarini L., Sathasivam K., Mahal A., Mott R., Seller M., and Bates G. P. (1997) Instability of highly expanded CAG repeats in mice transgenic for the Huntington's disease mutation. *Nat. Genet.* **15,** 197–200.

42. Trottier Y., Devys D., Imbert G., Saudou F., An I., Lutz Y., et al. (1995) Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nat. Genet.* **10,** 104–110.
43. Sapp E., Schwarz C., Chase K., Bhide P. G., Young A. B., Penney J., et al. (1997) Huntingtin localization in brains of normal and Huntington's disease patients. *Ann. Neurol.* **42,** 604–612.
44. Li S. H., Schilling G., Young W. S., 3d, Li X. J., Margolis R. L., Stine O. C., et al. (1993) Huntington's disease gene (IT15) is widely expressed in human and rat tissues. *Neuron* **11,** 985–993.
45. DiFiglia M., Sapp E., Chase K., Schwarz C., Meloni A., Young C., et al. (1995) Huntingtin is a cytoplasmic protein associated with vescicles in human an rat brain neurons. *Neuron* **14,** 1075–1081.
46. Sharp A. H., Love S. J., Schilling G., Li S. H., Li X. J., Bao J., et al. (1995) Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron* **14,** 1065–1074.
47. Bhide P. G., Day M., Sapp E., Schwarz C., Sheth A., Kim J., et al. (1996) Expression of normal and mutant huntigtin in the developing brain. *J. Neurosci.* **16,** 5523–5535.
48. Gourfinkel-An I., Cancel G., Trottier Y., Devys D., Tora L., Lutz Y., et al. (1997) Differential distribution of the normal and mutated forms of huntingtin in the human brain. *Ann. Neurol.* **42,** 712–719.
49. Fusco F. R., Chen Q., Lamoreaux W. J., Figueredo-Cardenas G., Jiao Y., Coffman J. A., et al. (1999) Cellular localization of huntingtin in striatal and cortical neurons in rat: lack correlation of with neuronal vulnerability in Huntington disease. *J. Neurosci.* **19,** 189–202.
50. Sharp A. H. and Ross C. A. (1996) Neurobiology of Huntington's disease. *Neurobiol. Dis.* **3,** 3–15.
51. Ona V. O., Li M., Vonsattel J. P., Andrews L. J., Khan S. Q., Chung W. M., et al. (1999) Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* **399,** 263–267.
52. Lin B., Rommens J. M., Graham R. K., Kalchman M., MacDonald H., Nasir J., et al. (1993) Differential 3′ polyadenylation of the Huntington disease gene results in two mRNA species with variable tissue expression. *Hum. Mol. Genet.* **2,** 1541–1545.
53. Schmitt I., Bachner D., Megow D., Henklein P., Hameister H., Epplen J. T., and Riess O. (1995) Expression of the Huntington disease gene in rodents: cloning the rat homologue and evidence for downregulation in non-neuronal tissues during development. *Hum. Mol. Genet.* **4,** 1173–1182.
54. Strong T. V., Tagle D. A., Valdes J. M., Elmer L. W., Boehm K., Swaroop M., et al. (1993) Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nat. Genet.* **5,** 259–265.
55. Syed V., Gomez E., and Hecht N. B. (1999) mRNAs encoding a von Ebner's-like protein and the Huntington disease protein are induced in rat male germ cells by Sertoli cells. *J. Biol. Chem.* **274,** 10737–10742.
56. Lin B., Nasir J., Kalchman M. A., McDonald H., Zeisler J., Goldberg Y. P., and Hayden M. R. (1995) Structural analysis of the 5′ region of mouse and human Huntington disease genes reveals conservation of putative promoter region and di- and trinucleotide polymorphisms. *Genomics* **25,** 707–715.
57. Holzmann C., Maueler W., Petersohn D., Schmidt T., Thiel G., Epplen J. T., and Riess O. (1998) Isolation and characterization of the rat huntingtin promoter. *Biochem. J.* **336,** 227–234.
58. Nasrin N., Buggs C., Kong X. F., Carnazza J., Goebl M., and Alexander-Bridges M. (1991) DNA-binding properties of the product of the testis-determining gene and a related protein. *Nature* **354,** 317–320.
59. Harley V. R., Jackson D. I., Hextall P. J., Hawkins J. R., Berkovitz G. D., Sockanathan S., Lovell-Badge R., and Goodfellow P. N. (1992) DNA binding activity of recombinant SRY from normal males and XY females. *Science* **255,** 453–456.
60. Coles R., Leggo J., and Rubinsztein D. C. (1997) Analysis of the 5′ upstream sequence of the Huntington's disease (HD) gene shows six new rare alleles which are unrelated to the age at onset of HD. *J. Med. Genet.* **34,** 371–374.
61. Coles R., Birdsall M., Wyttenbach A., and Rubinsztein D. C. (2000) 12-O-tetradecanoylphorbol-13-acetate down-regulates the huntingtin promoter at Spl sites. *Neuroreport* **11,** 3157–3161.
62. Hebb M. O., Denovan-Wright E. M., and Robertson H. A. (1999) Expression of the Huntington's disease gene is regulated in

astrocytes in the arcuate nucleus of the hypothalamus of postpartum rats. *FASEB J.* **13,** 1099–1106.
63. Hilditch-Maguire P., Trettel F., Passani L. A., Auerbach A., Persichetti F., and MacDonald M. E. (2000) Huntingtin: an iron-regulated protein essential for normal nuclear and perinuclear organelles. *Hum. Mol. Genet.* **9,** 2789–2797.
64. Schwarcz R., Whetsell W. O., Jr., and Mangano R. M. (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* **219,** 316–318.
65. Beal M. F., Kowall N. W., Ellison D. W., Mazurek M. F., Swartz K. J., and Martin J. B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **321,** 168–171.
66. Ferrante R. J., Kowall N. W., Cipolloni P. B., Storey E., and Beal M. F. (1993) Excitotoxin lesions in primates as a model for Huntington's disease: histopathologic and neurochemical characterization. *Exp. Neurol.* **119,** 46–71.
67. Alexi T., Borlongan C. V., Faull R. L., Williams C. E., Clark R. G., Gluckman P. D., and Hughes P. E. (2000) Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. *Progr. Neurobiol.* **60,** 409–470.
68. Brouillet E., Conde F., Beal M. F., and Hantraye P. (1999) Replicating Huntington's disease phenotype in experimental animals *Progr. Neurobiol.* **59,** 427–468.
69. Duyao M. P., Auerbach A. B., Ryan A., Persichetti F., Barnes G. T., McNeil S. M., et al. (1995) Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science* **269,** 407–410.
70. Nasir J., Floresco S. B., O'Kusky J. R., Diewert V. M., Richman J. M., Zeisler J., et al. (1995) Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* **81,** 811–823.
71. Zeitlin S., Liu J. P., Chapman D. L., Papaioannou V. E., and Efstratiadis A. (1995) Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat. Genet.* **11,** 155–162.
72. Dragatsis I., Efstratiadis A., and Zeitlin S. (1998) Mouse mutant embryos lacking huntingtin are rescued from lethality by wild-type extraembryonic tissues. *Development* **125,** 1529–1539.
73. O'Kusky J. R., Nasir J., Cicchetti F., Parent A., and Hayden M. R. (1999) Neuronal degeneration in the basal ganglia andloss of pallido subthalamic synapses in mice with targeted disruption of the Huntington's disease gene. *Brain Res.* **818,** 468–479.
74. Metzler M., Chen N., Helgason C. D., Graham R. K., Nichol K., McCutcheon K., et al. (1999) Life without huntingtin: normal differentiation into functional neurons. *J. Neurochem.* **72,** 1009–1018.
75. Hodgson J. G., Agopyan N., Gutekunst C. A., Leavitt B. R., LePiane F., Singaraja R., et al. (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* **23,** 181–192.
76. Levine M. S., Klapstein G. J., Koppel A., Gruen E., Cepeda C., Vargas M. E., et al. (1999) Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knock-in mouse models of Huntington's disease. *J. Neurosci. Res.* **58,** 515–532.
77. Metzler M., Helgason C. D., Dragatsis I., Zhang T., Gan L., Pineault N., et al. (2000) Huntingtin is required for normal hematopoiesis. *Hum. Mol. Genet.* **9,** 387–394.
78. White J. K., Auerbach W., Duyao M. P., Vonsattel J. P., Gusella J. F., Joyner A. L., and MacDonald M. E. (1997) Huntingtin is required for neurogenesis and is not impaired by Huntington's disease CAG expansion. *Nat. Genet.* **17,** 404–410.
79. Dragatsis I., Levine M. S., and Zeitlin S. (2000) Inactivation of hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nat. Genet.* **26,** 300–306.
80. Leavitt B. R., Guttman J., Hodgson G., Kimel G. H., Singaraja R., Vogl A. W., and Hayden M. R. (2001) Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo. *Am. J. Hum. Genet.* **68,** 313–324.
81. Mangiarini L., Sathasivam K., Seller M., Cozens B., Harper A., Hetherington C., et al. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87,** 493–506.
82. Cha J. H., Kosinski C. M., Kerner J. A., Alsdorf S. A., Mangiarini L., Davies S. W., et al. (1998) Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an

abnormal human huntington disease gene. *Proc. Natl. Acad. Sci. USA* **95,** 6480–6485.
83. Cha J. H., Frey A. S., Alsdorf S. A., Kerner J. A., Kosinski C. M., Mangiarini L., et al. (1999) Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **354,** 981–989.
84. Fain J. N., Del Mar N. A., Meade C. A., Reiner A., and Goldowitz D. (2001) Abnormalities in the functioning of adipocytes from R6/2 mice that are transgenic for the Huntington's disease mutation. *Hum. Mol. Genet.* **10,** 145–152.
85. Hulbert M. S., Zhou W., Wasmeier C., Kaddis F. G., Hutton J. C., and Freed C. R. (1999) Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes* **48,** 649–651.
86. Sathasivam K., Hobbs C., Turmaine M., Mangiarini L., Mahal A., Bertaux F., et al. (1999) Formation of polyglutamine inclusions in non-CNS tissue. *Hum. Mol. Genet.* **8,** 813–822.
87. Farrer L. A. (1985) Diabetes mellitus in Huntington disease *Clin. Genet.* **27,** 62–67.
88. Davies S. W., Turmaine M., Cozens B. A., DiFiglia M., Sharp A. H., Ross C. A., et al. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90,** 537–548.
89. Schilling G., Becher M. W., Sharp A. H., Jinnah H. A., Duan K., Kotzuk J. A., et al. (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum. Mol. Genet.* **8,** 397–407.
90. Yamamoto A., Lucas J. J., and Hen R. (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* **101,** 57–66.
91. Gossen M. and Bujard H. (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* **89,** 5547–5551.
92. Bates G. (2000) Huntington's disease. In reverse gear. *Nature* **404,** 944–945.
93. Orr H. T. and Zoghbi H. Y. (2000) Reversing neurodegeneration: a promise unfolds. *Cells* **101,** 1–4.
94. Ordway J. M., Tallaksen-Greene S., Gutekunst C. A., Bernstein E. M., Cearley J. A., Wiener H. W., et al. (1997) Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell* **91,** 753–763.
95. Cummings C. J. and Zoghbi H. Y. (2000) Fourteen and counting: unraveling trinucleotide repeat diseases. *Hum. Mol. Genet.* **9,** 909–916.
96. Reddy P. H., Williams M., Charles V., Garrett L., Pike-Buchanan L., Whetsell W. O., Jr., Miller G., and Tagle D. A. (1998) Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat. Genet.* **20,** 198–202.
97. Li H., Li S. H., Johnston H., Shelbourne P. F., and Li X. J. (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. *Nat. Genet.* **25,** 385–389.
98. Shelbourne P. F. (2000) Of mice and men: solving the molecular mysteries of Huntington's disease. *J. Anat.* **196,** 617–628.
99. Wheeler V. C., Auerbach W., White J. K., Srinidhi J., Auerbach A., Ryan A., et al. (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. *Hum. Mol. Genet.* **8,** 115–122.
100. Shelbourne P. F., Killeen N., Hevner R. F., Johnston H. M., Tecott L., Lewandoski M., et al. (1999) A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. *Hum. Mol. Genet.* **8,** 763–774.
101. Wheeler V. C., White J. K., Gutekunst C. A., Vrbanac V., Weaver M., Li X. J., et al. (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. *Hum. Mol. Genet.* **9,** 503–513.
102. Lin C. H., Tallaksen-Greene S., Chien W. M., Cearley J. A., Jackson W. S., Crouse A. B., et al. (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. *Hum. Mol. Genet.* **10,** 137–144.
103. Warrick J. M., Paulson H. L., Gray-Board G. L., Bui Q. T., Fischbeck K. H., Pittman R. N., and Bonini N. M. (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in Drosophila. *Cell* **93,** 939–949.
104. Jackson G. R., Salecker I., Dong X., Yao X., Arnheim N., Faber P. W., MacDonald M. E., and Zipursky S. L. (1998) Polyglutamine-expanded

human huntingtin transgenes induce degeneration of Drosophila photoreceptor neurons. *Neuron* **21,** 633–642.
105. Kazemi-Esfarjani P. and Benzer S. (2000) Genetic suppression of polyglutamine toxicity in Drosophila. *Science* **287,** 1837–1840.
106. Faber P. W., Alter J. R., MacDonald M. E., and Hart A. C. (1999) Polyglutamine-mediated dysfunction and apoptotic death of a Caenorhabditis elegans sensory neuron. *Proc. Natl. Acad. Sci. USA* **96,** 179–184.
107. Martindale D., Hackam A., Wieczorek A., Ellerby L., Wellington C., McCutcheon K., et al. (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat. Genet.* **18,** 150–154.
108. Cooper J. K., Schilling G., Peters M. F., Herring W. J., Sharp A. H., Kaminsky Z., et al. (1998) Truncated N-terminal fragments of huntingtin with expanded glutamine repeats form nuclear and cytoplasmic aggregates in cell culture. *Hum. Mol. Genet.* **7,** 783–790.
109. Hackam A. S., Singaraja R., Wellington C. L., Metzler M., McCutcheon K., Zhang T., Kalchman M., and Hayden M. R. (1998) The influence of huntingtin protein size on nuclear localization and cellular toxicity. *J. Cell Biol.* **141,** 1097–1105.
110. Liu Y. F. (1998) Expression of polyglutamine-expanded huntingtin activates the SEK1-JNK pathway and induces apoptosis in a hippocampal neuronal cell line. *J. Biol. Chem.* **273,** 28873–28877.
111. Liu Y. F., Dorow D., and Marshall J. (2000) Activation of MLK2-mediated signaling cascades by polyglutamine-expanded huntingtin. *J. Biol. Chem.* **275,** 19,035–19,040.
112. Kim M., Lee H. S., LaForet G., McIntyre C., Martin E. J., Chang P., et al. (1999) Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J. Neurosci.* **19,** 964–973.
113. Wainwright M. S., Perry B. D., Won L. A., O'Malley K. L., Wang W. Y., Ehrlich M. E., and Heller A. (1995) Immortalized murine striatal neuronal cell lines expressing dopamine receptors and cholinergic properties. *J. Neurosci.* **15,** 676–688.
114. Saudou F., Finkbeiner S., Devys D., and Greenberg ME. (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* **95,** 55–66.
115. Moulder K. L., Onodera O., Burke J. R., Strittmatter W. J., Johnson E. M., Jr. (1999) Generation of neuronal intranuclear inclusions by polyglutamine-GFP: analysis of inclusion clearance and toxicity as a function of polyglutamine length. *J. Neurosci.* **19,** 705–715.
116. Sanchez I., Xu C. J., Juo P., Kakizaka A., Blenis J., Yuan J. (1999) Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* **22,** 623–633.
117. Li S. H., Cheng A. L., Li H., and Li X. J. (1999) Cellular defects and altered gene expression in PC12 cells stably expressing mutant huntingtin. *J. Neurosci.* **19,** 5159–5172.
118. Greene L. A. and Tischler A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.
119. Lunkes A. and Mandel J. L. (1998) A cellular model that recapitulates major pathogenic steps of Huntington's disease. *Hum. Mol. Genet.* **7,** 1355–1361.
120. Cattaneo E., Magrassi L., Butti G., Santi L., Giavazzi A., and Pezzotta S. (1994) A short term analysis of the behaviour of conditionally immortalized neuronal progenitors and primary neuroepithelial cells implanted into the fetal rat brain. *Brain Res. Dev. Brain Res.* **83,** 197–208.
121. Cattaneo E., De Fraja C., Conti L., Reinach B., Bolis L., Govoni S., and Liboi E. (1996) Activation of the JAK/STAT pathway leads to proliferation of ST14A central nervous system progenitor cells. *J. Biol. Chem.* **271,** 23,374–23,379.
122. Cattaneo E. and Conti L. (1998) Generation and characterization of embryonic striatal conditionally immortalized ST14A cells. *J. Neurosci. Res.* **15,** 223–234.
123. Lundberg C., Martinez-Serrano A., Cattaneo E., McKay R. D., and Bjorklund A. (1997) Survival, integration, and differentiation of neural stem cell lines after transplantation to the adult rat striatum. *Exp. Neurol.* **145,** 342–360.
124. Benedetti S., Pirola B., Pollo B., Magrassi L., Bruzzone M. G., Rigamonti D., et al. (2000) Gene therapy of experimental brain tumors using neural progenitor cells. *Nat. Med.* **6,** 447–450.

125. Rigamonti D., Bauer J. H., De-Fraja C., Conti L., Sipione S., Sciorati C., et al. (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J. Neurosci.* **20,** 3705–3713.
126. Ehrlich M., Conti L., Toselli M., Taglietti L., Fiorillo E., Taglietti V., et al. (2000) ST14A cells have properties of a medium size spiny neuron. *Exp. Neurol.,* **167,** 215–226.
127. Varani K., Rigamonti D., Sipione S., Camurri A. Borea P., Cattabeni F., Abbracchio M. P., and Cattaneo E. (2001) Aberrant amplification of A2A receptor signalling in striatal cells expressing mutant huntingtin. *FASEB J.,* **15,** 1245–1247.
128. Sawa A., Wiegand G. W., Cooper J., Margolis R. L., Sharp A. H., Lawler J. F., Jr., et al. (1999) Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Nat. Med.* **5,** 1194–1198.
129. Wheeler V. C., White J. K., Gutekunst C. A., Vrbanac V., Weaver M., Li X. J., et al. (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. *Hum. Mol. Genet.* **9,** 503–513.
130. Trettel F., Rigamonti D., Hilditch-Maguire P., Wheeler V. C., Sharp A. H., Persichetti F., Cattaneo E., and MacDonald M. E. (2000) Dominant phenotypes produced by the HD mutation in STHdh (Q111) striatal cells. *Hum. Mol. Genet.* **9,** 2799–2809.
131. Zoghbi H. Y. and Orr H. T. (1999) Polyglutamine diseases: protein cleavage and aggregation. *Curr. Opin. Neurobiol.* **9,** 566–570.
132. Perutz M. F., Johnson T., Suzuki M., and Finch J. T. (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA* **91,** 5355–5358.
133. Kahlem P., Terre C., Green H., and Djian P. (1996) Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: relevance to diseases of the nervous system. *Proc. Natl. Acad. Sci. USA* **93,** 14580–14585.
134. DiFiglia M., Sapp E., Chase K. O., Davies S. W., Bates G. P., Vonsattel J. P., and Aronin N. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* **277,** 1990–1993.
135. Becher M. W., Kotzuk J. A., Sharp A. H., Davies S. W., Bates G. P., Price D. L., and Ross C. A. (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. *Neurobiol. Dis.* **4,** 387–397.
136. Gutekunst C. A., Li S. H., Yi H., Mulroy J. S., Kuemmerle S., Jones R., et al. (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J. Neurosci.* **19,** 2522–2534.
137. Reddy P. H., Charles V., Williams M., Miller G., Whetsell W. O., Jr., and Tagle D. A. (1999) Transgenic mice expressing mutated full-length HD cDNA: a paradigm for locomotor changes and selective neuronal loss in Huntington's disease. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **354,** 1035–1045.
138. Ross C. A. (1997) Intranuclear neuronal inclusions: a common pathogenic mechanism for glutamine-repeat neurodegenerative diseases. *Neuron* **19,** 1147–1150.
139. Sisodia S. (1998) Nuclear inclusion in glutamine repeat disorders: Are they pernicious, coincidental, or beneficial? *Cell* **95,** 1–4.
140. Chai Y., Koppenhafer S. L., Bonini N. M., and Paulson H. L. (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. *J. Neurosci.* **19,** 10,338–10,347.
141. Carmichael J., Chatellier J., Woolfson A., Milstein C., Fersht A. R., and Rubinsztein D. C. (2000) Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **97,** 9701–9705.
142. Jana N. R., Tanaka M., Wang G. h., and Nukina N. (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. *Hum. Mol. Genet.* **9,** 2009–2018.
143. Wyttenbach A., Carmichael J., Swartz J., Furlong R. A., Narain Y., Rankin J., and Rubinsztein D. C. (2000) Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **97,** 2898–2903.
144. Gabai V. L., Meriin A. B., Yaglom J. A., Volloch V. Z., and Sherman M. Y. (1998) Role of Hsp70

in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Lett.* **438,** 1–4.
145. Nagai Y., Tucker T., Ren H., Kenan D. J., Henderson B. S., Keene J. D., Strittmatter W. J., and Burke J. R. (2000) Inhibition of polyglutamine protein aggregation and cell death by novel peptides identified by phage display screening. *J. Biol. Chem.* **275,** 10,437–10,442.
146. Tran P. B. and Miller R. J. (1999) Aggregates in neurodegenerative disease: crowds and power. *Trends Neurosci.* **22,** 194–197.
147. Li X. J., Li S. H., Sharp A. H., Nucifora F. C., Jr., Schilling G., Lanahan A., et al. (1995) A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* **378,** 398–402.
148. Wanker E. E., Rovira C., Scherzinger E., Hasenbank R., Walter S., Tait D., Colicelli J., and Lehrach H. (1997) HIP-1 a huntingtin interacting protein isolated by the yeast two hybrid system. *Hum. Mol. Genet.* **6,** 487–495.
149. Kalchman M. A., Koide H. B., McCutcheon K., Graham R. K., Nichol K., Nishiyama K., et al. (1997) HIP1, a human homologue of S. cerevisiae Sla2p, interacts with membrane-associated huntingtin in the brain. *Nat. Genet.* **16,** 44–53.
150. Sittler A., Walter S., Wedemeyer N., Hasenbank R., Scherzinger E., Eickhoff H., et al. (1998) SH3GL3 associates with the huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. *Mol. Cell* **2,** 427–436.
151. Hackam A. S., Yassa A. S., Singaraja R., Metzler M., Gutekunst C. A., Gan L., et al. (2000) Huntingtin interacting protein 1 induces apoptosis via a novel caspase-dependent death effector domain. *J. Biol. Chem.* **275,** 41,299–41,308.
152. Li S. H., Li H., Torre E. R., and Li X. J. (2000) Expression of huntingtin-associated protein-1 in neuronal cells implicates a role in neurite growth. *Mol. Cell. Neurosci.* **16,** 168–183.
153. Bertaux F., Sharp A. H., Ross C. A., Lehrach H., Bates G. P., and Wanker E. (1998) HAP1-huntingtin interactions do not contribute to the molecular pathology in Huntington's disease transgenic mice. *FEBS Lett.* **426,** 229–232.
154. Faber P. W., Barnes G. T., Srinidhi J., Chen J., Gusella J. F. and MacDonald M. E. (1998) Huntingtin interacts with a family of WW domain proteins. *Hum. Mol. Genet.* **7,** 1463–1474.
155. Boutell J. M., Thomas P., Neal J. W., Weston V. J., Duce J., Harper P. S., and Jones L. A. (1999) Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Hum. Mol. Gene.* **8,** 1647–1655.
156. Steffan J. S., Kazantsev A., Spasic-Boskovic O., Greenwald M., Zhu Y. Z., Gohler H., et al. (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. USA* **97,** 6763–6768.
157. Einbond A. and Sudol M. (1996) Towards prediction of cognate complexes between the WW domain and proline-rich ligands. *FEBS Lett.* **384,** 1–8.
158. Sudol M., Chen H. I., Bougeret C., and Einbond A. (1995) Characterization of a novel protein-binding module: the WW domain. *FEBS Lett.* **369,** 67–71.
159. Liu F. Y., Deth R. C., and Devys D. (1997) SH3 domain-dependent association of huntingtin with epidermal growth factor receptor signaling complex. *J. Biol. Chem.* **272,** 8121–8124.
160. Bao J., Sharp A. H., Wagster M. V., Becher M., Schilling G., Ross C. A., Dawson V. L., and Dawson T. M. (1996) Expansion of polyglutamine repeat in huntingtin leads to abnormal protein interactions involving calmodulin. *Proc. Natl. Acad. Sci. USA* **93,** 5037–5042.
161. Boutell J. M., Wood J. D., Harper P. S., and Jones A. L. (1998) Huntingtin interacts with cystathionine β-synthase. *Hum. Mol. Genet.* **7,** 371–378.
162. Burke J. R., Enghild J. J., Martin M. E., Jou Y. S., Myers R. M., Roses A. D., Vance J. M., and Strittmatter W. J. (1996) Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nat. Med.* **2,** 347–350.
163. Kalchman M. A., Graham R. K., Xia G., Koide H. B., Hodgson J. G. Graham K. C., et al. (1996) Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J. Biol. Chem.* **271,** 19385–19394.
164. Peters M. F. and Ross C. A. (2000) Isolation of a 40 kDa huntingtin-associated protein. *J. Biol. Chem.,* **276,** 3188–3194.
165. Beal M. F. (2000) Energetics in the pathogenesis of neurodegenerative diseases. *Trends Neurosci.* **23,** 298–304.
166. Cha J. H. (2000) Transcriptional dysregulation in Huntington's disease. *Trends Neurosci.* **23,** 387–392.

167. Velier J., Kim M., Schwarz C., Kim T. W., Sapp E., Chase K., Aronin N., and DiFiglia M. (1998) Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways. *Exp. Neurol.* **152,** 34–40.
168. Kim M., Velier J., Chase K., Laforet G., Kalchman M. A., Hayden M. R., et al. (1999) Forskolin and dopamine D1 receptor activation increase huntingtin's association with endosomes in immortalized neuronal cells of striatal origin. *Neuroscience* **89,** 1159–1167.
169. Block-Galarza J., Chase K. O., Sapp E., Vaughn K. T., Vallee R. B., DiFiglia M., and Aronin N. (1997) Fast transport and retrograde movement of huntingtin and HAP 1 in axons. *Neuroreport* **8,** 2247–2251.
170. Hirakura Y., Azimov R., Azimova R., and Kagan B. L. (2000) Polyglutamine-induced ion channels: a possible mechanism for the neurotoxicity of Huntington and other CAG repeat diseases. *J. Neurosci. Res.* **60,** 490–494.
171. Kegel K. B., Kim M., Sapp E., McIntyre C., Castano J. G., Aronin N., and DiFiglia M. (2000) Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *J. Neurosci.* **20,** 7268–7278.
172. Gerber H. P., Seipel K., Georgiev O., Hofferer M., Hug M., Rusconi S., and Schaffner W. (1994) Transcriptional activation modulated by homopolymeric glutamine and proline stretches. *Science* **263,** 808–811.
173. Passani L. A., Bedford M. T., Faber P. W., McGinnis K. M., Sharp A. H., Gusella J. F., Vonsattel J. P., and MacDonald M. E. (2000) Huntingtin's WW domain partners in Huntington's disease post-mortem brain fulfill genetic criteria for direct involvement in Huntington's disease pathogenesis. *Hum. Mol. Genet.* **9,** 175–182.
174. Andrade M. A. and Bork P. (1995) HEAT repeats in the Huntington's disease protein. *Nat. Genet.* **11,** 115–116.
175. Vetter I. R., Arndt A., Kutay U., Gorlich D., and Wittinghofer A. (1999) Structural view of the Ran-Importin beta interaction at 2.3 A resolution. *Cell* **97,** 635–646.
176. Bessert D. A., Gutridge K. L., Dunbar J. C., and Carlock L. R. (1995) The identification of a functional nuclear localization signal in the Huntington disease protein. *Brain Res. Mol. Brain Res.* **33,** 165–173.
177. Li S. H., Lam S., Cheng A. L., and Li X. J. (2000) Intranuclear huntingtin increases the expression of caspase-1 and induces apoptosis. *Hum. Mol. Genet.* **9,** 2859–2867.
178. Klivenyi P., Ferrante R. J., Matthews R. T., Bogdanov M. B., Klein A. M., Andreassen O. A., et al. (1999) Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med.* **5,** 347–350.
179. Friedlander R. M., Brown R. H., Gagliardini V., Wang J., and Yuan J. (1997) Inhibition of ICE slows ALS in mice. *Nature* **388,** 31–35.
180. Hara H., Fink K., Endres M., Friedlander R. M., Gagliardini V., Yuan J., and Moskowitz M. A. (1997) Attenuation of transient focal cerebral ischemic injury in transgenic mice expressing a mutant ICE inhibitory protein. *J. Cereb. Blood Flow Metab.* **17,** 370–375.
181. Chen M., Ona V. O., Li M., Ferrante R. J., Fink K. B., Zhu S., et al. (2000) Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat. Med.* **6,** 797–801.
182. Goldberg Y. P., Nicholson D. W., Rasper D. M., Kalchman M. A., Koide H. B., Graham R. K., et al. (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat. Genet.* **13,** 442–449.
183. Wellington C. L., Ellerby L. M., Hackam A. S., Margolis R. L., Trifiro M. A., Singaraja R., et al. (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.* **273,** 9158–9167.
184. Wellington C. L., Singaraja R., Ellerby L., Savill J., Roy S., Leavitt B., et al. (2000) Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and non-neuronal cells. *J. Biol. Chem.* **275,** 19831–19838.
185. Miyashita T., Matsui J., Ohtsuka Y., Mami U., Fujishima S., Okamura-Oho Y., Inoue T., and Yamada M. (1999) Expression of extended polyglutamine sequentially activates initiator and effector caspases. *Biochem. Biophys. Res. Commun.* **257,** 724–730.
186. Nagata K., Driessens M., Lamarche N., Gorski J. L., and Hall A. (1998) Activation of G1 progression, JNK mitogen-activated protein kinase, and actin filament assembly by the exchange factor FGD1. *J. Biol. Chem.* **273,** 15,453–15,457.
187. Tournier C., Whitmarsh A. J., Cavanagh J., Barrett T., and Davis R. J. (1997) Mitogen-activated protein kinase 7 is an activator of the c-

Jun NH2-terminal kinase. *Proc. Natl. Acad. Sci. USA* **94,** 7337–7342.
188. Browne S. E., Bowling A. C., MacGarvey U., Baik M. J., Berger S. C., Muqit M. M., Bird E. D., and Beal M. F. (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann. Neurol.* **41,** 646–653.
189. Panov A. V., Burke J. R., Strittmatter W. J., and Greenmayre (2000) The mitochondrial permeability transition in Huntington's Disease. Abstract 182.4, 30th Annual Meeting, Society for Neuroscience, New Orleans, U.S.A.
190. Clarke G., Collins R. A., Leavitt B. R., Andrews D. F., and Hayden M. R., Lumsden C. J., and McInnes R. R. (2000) A one-hit model of cell death in inherited neuronal degenerations. *Nature* **406,** 195–199.
191. Sapp E., Penney J., Young A., Aronin N., Vonsattel J. P., and DiFiglia M. (1999) Axonal transport of N-terminal huntingtin suggests early pathology of corticostriatal projections in Huntington's disease. *J. Neuropathol. Exp. Neurol.* **58,** 165–173.
192. Zuccato C., Ciammola A., Rigamonti D., Leavitt B. R., Goffredo D., Conti L., MacDonald M. E., Friedlander R. M., Silani V., Hayden M. R., Timmusk T., Sipione S., and Cattaneo E. (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* **293,** 493–498.
193. Nucifora F. C., Sasaki M., Peters M. F., Huang H., Cooper J. K., Yamada M., Takahashi H., Tsuji S., Troncoso J., Dawson V. L., Dawson T. M., and Ross C. A. (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291,** 2423–2428.