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Huntington's Disease and Dentatorubral-Pallidoluysian Atrophy: Proteins, Pathogenesis and Pathology

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Each of the glutamine repeat neurodegenerative diseases has a particular pattern of pathology largely restricted to the CNS. However, there is considerable overlap among the regions affected, suggesting that the diseases share pathogenic mechanisms, presumably involving the glutamine repeats. We focus on Huntington's disease (HD) and Dentatorubral-pallidoluysian atrophy (DRPLA) as models for this family of diseases, since they have striking similarities and also notable differences in their clinical features and pathology, . We review the pattern of pathology in adult and juvenile onset cases. Despite selective pathology, the disease genes and their protein products (huntingtin and atrophin-1) are widely expressed. This presents a central problem for all the glutamine repeat diseases--how do widely expressed gene products give rise to restricted pathology? The pathogenic effects are believed to occur via a "gain of function" mechanism at the protein level. Mechanisms of cell death may include excitotoxicity, metabolic toxicity, apoptosis, and free radical stress. Emerging data indicate that huntingtin and atrophin-1 may have distinct protein interactions. The specific interaction partners may help explain the selective pathology of these diseases.

Introduction

The remarkable patterns of degeneration and neuronal selectivity in the glutamine repeat neurodegenerative diseases have been intriguing investigators since the earliest microscopic descriptions. Within the CNS each disease has a distinctive pattern of neurodegeneration, but the regions affected show considerable overlap (9, 49, 71, 77, 88, 102, 131). Thus, there appears to be a group of glutamine repeat sensitive brain regions. These include the striatum, globus pallidus, subthalamic and red nuclei, basis pontis, brainstem motor nuclei, cerebellar Purkinje cells and dentate nucleus, and spinal motor neurons. By contrast, there are a number of other neurodegenerative disorders which devastate brain regions that are relatively spared in all the known glutamine repeat disorders For example, the hippocampus and the nucleus basalis of Meynert, which are affected in Alzheimer's disease, are intact in the glutamine repeat disorders. The explanation for this pattern of distinct, but overlapping, areas of vulnerability in this disease family remains unknown.

All of the glutamine repeat diseases are believed to exert their pathogenic effects via a "gain of function" mechanism at the protein level. The protein products of each of the disease genes appear to have relatively widespread distributions of expression. Nevertheless, pathology in each of the diseases is largely restricted to the central nervous system. How widely expressed genes cause selective pathology remains a central mystery. In this chapter we will focus on two of the disorders as models, HD and DRPLA. We will examine their morphologic variability and similarities; review possible important intrinsic cellular toxicity pathways such as excitotoxicity; and, finally, present recent developments in our understanding of protein-protein interactions in HD and DRPLA.

Neuronal Selectivity in HD and DRPLA

How do the glutamine repeat gene products cause neuronal degeneration? The answer to this question remains unknown. However, one could imagine two general possibilities which are not necessarily mutually exclusive. The first is that the glutamine repeat expansion by itself leads to some kind of toxic effect within cells. The protein containing the glutamine repeat would only modify the glutamine repeat toxicity. This model emphasizes the commonalities among the diseases, and suggests that when the glutamine repeat is long enough in any of the disease proteins, the common toxic effect should predominate and the pattern of pathology would be severe and would be similar for all

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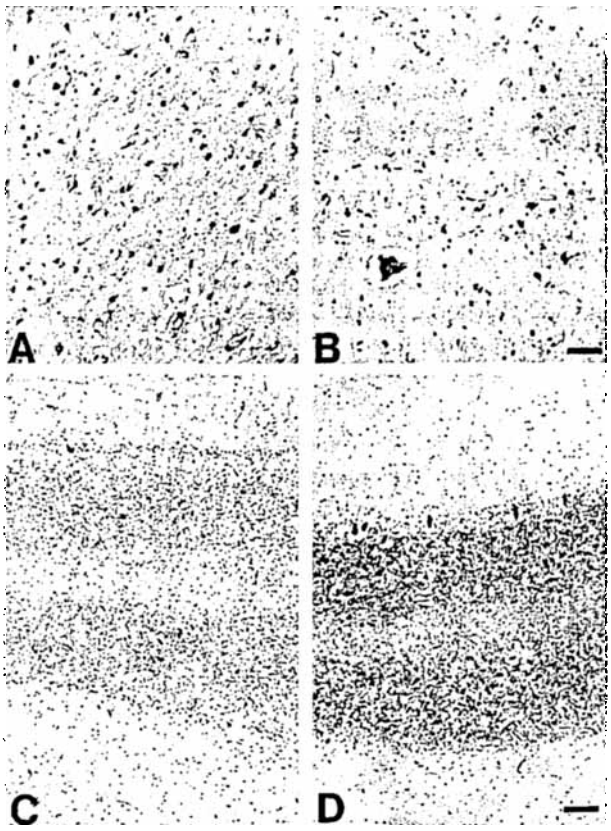

Figure 1. Similarities and differences in the pattern of neurodegeneration in juvenile onset HD and DRPLA. A,B) Striking striatal degeneration with prominent astrocytosis and neuronal loss, the hallmark of adult and juvenile onset HD, is present in the putamen of a juvenile onset HD case (A) but not found in juvenile onset DRPLA (B). C,D) Marked neuronal depopulation with loss of Purkinje and granular cells (C) is typical of juvenile onset HD, but only rarely seen in adult onset HD. Juvenile onset DRPLA (D) has less severe neuronal loss in the cerebellar folia, but focally Purkinje cell loss is nearly complete. Hematoxylin and Eosin, bars: A,B = 50 _m; C,D = 100 _m.

of the diseases.

The second possibility is that for each disease, the expanded glutamine repeat alters the particular protein product in a specific way, and depending on the role of the particular protein product within cells, this conformational change would activate a unique pathway of toxicity. This would emphasize the differences among the protein products and predict that no matter how long the glutamine repeat, each disease should give rise to a distinct pattern of pathology. Given the complexity of neurological disease pathogenesis, both viewpoints might be valid.

Clinical and morphological differences are found when one compares juvenile onset cases to adult onset cases of HD and DRPLA. Thus, mutations of the same gene can produce varied enough mediators of pathogenesis to alter both the clinical expression of disease and the morphological phenotype of neurodegeneration. Study of the juvenile cases may give some clues to help address the question posed above. That is, if there were a single unitary mechanism of glutamine repeat toxicity, juvenile cases (which arise when the glutamine repeats are very long for all the diseases) would tend to have identical patterns of pathology no matter which disease one studied. By contrast, if the mechanism of pathogenesis were distinct for the different diseases, then one would expect the juvenile cases to maintain a distinct pattern of pathology.

Morphologic variability and similarities in Adult HD and DRPLA. Despite their remarkably similar clinical phenotypes, the primary sites of neurodegeneration are different in adult onset HD and DRPLA. Briefly, HD is characterized by the loss of striatonigral and striatopallidal neurons accompanied by loss of striatal neuropil, development of reactive astrocytic changes, and less prominent degeneration of the globus pallidus (3, 91, 110, 127). Variable degenerative features have been found in the subthalamic nucleus, claustrum, pons, olivary complex, amygdala, neocortex, and Purkinje cell population of the cerebellum in HD cases (46, 59, 98, 127). Pathologic changes in these areas consist of astrocytosis with or without appreciable mild neuronal loss. Rare cases of HD have been found to have marked Purkinje cell loss (98). The dentate nucleus of the cerebellum is rarely mentioned in reports of HD in the classic literature but it is generally recognized that minimal, if any, neuronal loss in this nucleus is typical of HD.

Adult DRPLA, although clinically similar to HD, is primarily the result of degeneration of the cerebellar dentate nucleus and globus pallidus with disruption of the dentatofugal and pallidofugal systems (15, 39, 54, 87, 113, 119, 129). Neuronal loss in the dentate can be quite severe with astrocytosis and distortion of the dentate ribbon. Variable degrees of neuronal loss are found in the globus pallidus, red nucleus, and subthalamic (corpus Luys) nucleus. Some reports have stressed a differential involvement of the external segment of the globus pallidus over the internal segment, but this appears to be quite variable. White matter degeneration is a prominent feature of DRPLA, especially in the hilum of the dentate nucleus and adjacent deep cerebellar white matter. Focal cerebral white matter spongiosis can be found in the corona radiata (15, 87, 129, 130). Reactive astrocytosis without identifiable neuronal loss can be found in the thalamus (15, 36), striatum (87,

119), and basis pontis (54). Neuroaxonal dystrophy (axonal swellings and neuronal loss) in the nucleus gracilis can be found in many cases (36, 39, 130) and olivary complex degeneration or "olivary hypertrophy" can also be identified (36, 87). Thus, areas of overlap between adult HD and DRPLA center on the involvement of the globus pallidus with minor reactive changes in common in the subthalamic nucleus and thalamus.

Juvenile HD and DRPLA. The clinical features of juvenile HD and juvenile DRPLA are often different compared to their adult counterparts. Juvenile onset HD frquently has morerigidity rather than chorea. Seizures may be present. Juvenile onset DRPLA is also often characterized by seizures, but without rigidity. Morphologically, there are also significant differences in that pediatric cases of both disorders have more widespread degeneration than typical adult cases. Pediatric HD is a relatively rare entity, estimated to be less than 1 or 2% of all HD cases (22, 23, 62, 89, 98). The caudate and putamen (Figure 1) show marked neuronal loss and prominent astrocytosis like adult HD cases, but in addition, many cases have widespread cerebellar atrophy with loss of both Purkinje and granular cells (Figure 1). The globus pallidus is markedly atrophic, although it has been reported to contain the normal number of neurons (23). Astrocytosis in the globus pallidus has been found to be greater in the external segment than the internal segment. Cases of juvenile HD have also been reported to have astrocytosis or varying degrees of neuronal loss in the dentate nucleus of the cerebellum (Figure 2), thalamus, hippocampus including dentate gyrus, and lateral vestibular nuclei (23). Many cases have appreciable neocortical neuronal loss, including focal laminar necrosis. Interestingly, numerous axonal spheroids have been noted in the nucleus gracilis in juvenile HD cases, a finding typical in the DRPLA cases originally reported as the Haw River syndrome in the United States (20, 23, 36) and other adult and juvenile DRPLA cases (15, 39).

DRPLA has been extremely well studied in the Japanese population where the differences between juvenile onset and adult onset cases were first recognized through the efforts of several investigators to establish clinical subtypes of DRPLA (54, 121, 125). Although the pattern of neurodegeneration in adult DRPLA is quite variable, it appears that juvenile DRPLA cases (Figure 2) have more widespread neuronal loss and astrocytosis (in addition to dentate nucleus degeneration) when compared to adult DRPLA cases. These changes have been described in the striatum, thal-

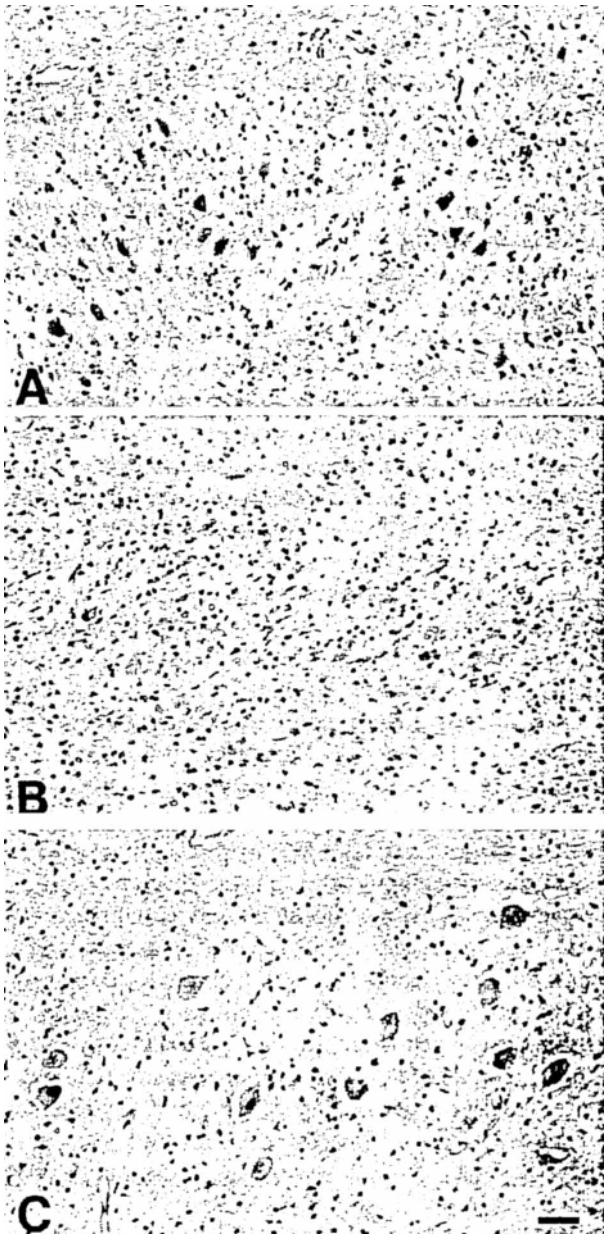

Figure 2. Dentate nucleus of the cerebellum in juvenile onset HD and DRPLA. A-C) Only mild neuronal loss and no appreciable astrocytosis is present in the dentate nucleus of juvenile onset HD (A) compared to the marked depopulation of the dentate found in juvenile DRPLA (B). Remaining neurons in the juvenile HD dentate nucleus (A) have degenerative and atrophic features when compared to those of a normal subject (C). Also present in the juvenile DRPLA case, and typical of most DRPLA cases regardless of age of onset, is prominent astrocytosis and marked disruption of the dentate ribbon (B) not found in juvenile HD (A) or normal subject (C). Hematoxylin and Eosin, bar = 50 _m.

amus and cerebral cortex (87, 119, 121).

In both juvenile HD and juvenile DRPLA there is a relationship between the length of the CAG triplet repeat and the age of onset. It has been shown that juvenile onset cases have larger triplet repeat expansions than adult onset cases (35, 56, 67, 116). Thus, the morphological differences between juvenile and adult onset cases are, at least in part, correlated with the triplet repeat length, although the pathogenetic mechanism remains unknown (56, 67, 114).

The markedly greater severity of the juvenile onset cases provides a further opportunity to explore similarities and differences in the pathogenesis of HD and DRPLA. The data appear to support both of the two possibilities proposed above. The juvenile cases of both diseases have Purkinje cell degeneration (like SCA1), consistent with a common "glutamine repeat" pathogenic process. However, the striatum is relatively spared in juvenile DRPLA, and the dentate nucleus of the cerebellum is relatively spared in juvenile HD, indicating that there must also be differences in pathogenesis.

Neuronal Morphology in HD. While there has been a great deal of attention paid to the patterns of neuronal loss in HD and the other glutamine repeat diseases, there has been less attention focused on the morphology of the surviving neurons. Perhaps one might expect that these neurons would be atrophic and shrunken. However, surprisingly, this is not necessarily what one sees. Studies of cell morphology have been conducted using the Golgi method in both caudate and cortex from HD cases (40, 41, 115). In both cases, HD neurons showed evidence of "plastic" or "regenerative" changes. These included an increased number of dendrites, increased long recurved dendrites, and increased density and size of spines on dendrites. In addition, surviving HD cells may have a greater somatic area and a greater number of dendrites.

The changes appear to occur in two "sequential but overlapping phases" described by Graveland et al. (41) as follows: "an earlier reactive state involves the growth of dendrites (long recurved endings, appendages and increased density and size of spines). As the disease progresses degenerative events characterized by a loss of spines and dendritic segments predominate." Graveland et al. (41) suggested "that neurons are responding to abnormal cues from the internal or external milieu of the cell. A metabolic disturbance in the neuron, perhaps under the control of the HD gene, may lead to dendritic hyperplasia."

Cellular Toxicity

Beginning long before the identification of the HD gene (52) animal model studies had implicated several mechanisms of cell death in HD. Most fully developed are studies of excitotoxicity and metabolic toxicity.

Excitotoxicity. Excitotoxicity has been a model of cell death in the striatum in HD for over 20 years (29,82). The striatum receives dense excitatory glutamatergic input from the cortex. Excitotoxic lesions of the striatum in animals produced by infusion of glutamate or ionotropic glutamate receptor agonists reproduce many of the neuropathological changes seen in HD. N-Methyl-D-aspartate (NMDA) receptor agonists, such as quinolinic acid, produce the best replica of HD-like changes, including gliosis and selective loss of medium spiny gamma-aminobutyric acid (GABA)ergic projection neurons with relative sparing of large somatostatin, neuropeptide Y, and cholinergic interneurons (10,12,134). Medium spiny neurons in the striata of quinoline acid-lesioned rats also show changes in neurite morphology and Calbindin D28K (a Ca2+ binding protein) immunoreactivity similar to those seen in HD brains (50). In addition, the ablation of corticostriatal glutamatergic projections before treatment with glutamate agonists attenuates striatal excitotoxicity in this model (82). These findings suggest a possible role for NMDA receptor-mediated excitotoxicity in HD.

One puzzle regarding the role of NMDA receptors in cell death in HD is the relative distribution of pathology of HD compared to NMDA receptors (27, 83, 84, 135). NMDA receptors are equally dense in neocortex, striatum, and hippocampus, as well as other regions, whereas the pathology of HD is striking in the striatum, but the hippocampus is relatively unaffected. Furthermore, within the cortex, neurodegeneration in HD is most prominent in deep layers, whereas NMDA receptors are most dense in superficial layers. Therefore, even if ionotropic glutamate receptors are involved, other factors are also likely to contribute to cell death in HD. The exact mechanisms of cell death resulting from the excessive activation of ionotropic excitatory amino acids are not yet clear. Cell culture models show two temporal phases of neural cell death after exposure to toxic levels of excitatory amino acid or agonist: an acute phase in which cells swell and die by osmotic lysis, and a delayed phase, requiring hours to days (24, 25). Prevention of the acute phase does not prevent the delayed phase, which more closely resembles in vivo excitotoxicity. The delayed phase requires at least a transient increase in intracellular Ca2+ channels.

Increased intracellular Ca2+ leads to the activation of a number of cellular processes that may be involved in cell death, including the activation of protein kinases, proteases, and phospholipases and the synthesis of nitric oxide. Excitotoxicity can cause cells to die by either necrosis or apoptosis, but the delayed phase is more likely to be apoptotic.

Metabolic Toxicity. Another animal model that produces a pattern of pathology quite similar to that of HD involves the subacute disruption of energy metabolism by mitochondrial toxins, such as malonate and 3-nitropropionic acid (3NPA) (11-13,19). Ingestion of 3NPA via contaminated food produced selective lesions of the basal ganglia in humans (76). Systemic injection of this toxin in rats produces selective loss of medium spiny neurons in the striatum. Neurotoxic effects can be blocked by prior decortication or, in vitro, by antagonists of excitatory amino acids. Lesions produced by the mitochondrial toxin malonate are blocked by Coenzyme Q10 and nicotinamide, consistent with a mechanism involving energy depletion (14). Chronic 3NPA treatment in baboons replicates cognitive and motor deficits of HD and causes selective bilateral lesions of the striatum (92). These results suggest that a mild impairment of mitochondrial energy metabolism might selectively increase the vulnerability of medium spiny striatal neurons to excitotoxicity (3, 43).

Consistent with a role for mitochondria, a number of neurodegenerative diseases with pathology in the basal ganglia are caused by mitochondrial DNA mutations. These include Leber's optic atrophy, mitochondrial encephalopathy with lactic acidosis and stroke-like symptoms (MELAS), and myoclonic epilepsy-ragged red fiber disease (MERFF) (13). Mitochondrial heterogeneity due to tissue-specific isoenzyme forms of respiratory chain complexes might account for the loss of differing groups of neurons in different diseases (13, 26, 111).

Free radicals have been implicated in the excitotoxic model and metabolic toxicity model of HD in rats (28, 34, 106, 107). Pretreatment of rats with free radical spin traps attenuated lesions produced by the intrastriatal injection of glutamate agonists.

One free radical that may be involved in excitotoxicity is nitric oxide, the product of the enzyme nitric oxide synthase (NOS) (18, 30). In cell cultures, nitric oxide is a mediator of NMDA toxicity (30). Treatment with NOS inhibitors ameliorates several forms of excitotoxicity in animal models (107). Neurons from mice with deletions of the neuronal NOS gene are resistant to both excitotoxicity (31) and metabolic toxicity (108).

Recent evidence from studies of HD patients is also consistent with energy defects. Magnetic resonance spectroscopy can detect evidence of altered energy metabolism in HD patients (60, 61) comparable to similar evidence in rats using excitotoxic lesions (61). Other magnetic resonance spectroscopy studies suggest the possibility of increased glutamate in the striatum of patients with early HD (120). Furthermore, recent autopsy studies have indicated neurochemical defects in several mitochondrial enzyme complexes in postmortem brain tissue from patients with HD (44).

An interesting possibility to help explain the heterogeneity of pathology is the possibility that interference with energy metabolism at different points can cause different patterns of pathology. As discussed above, 3NPA and malonate, which are inhibitors of complex II in the mitochondrial electron transport chain, cause selective neuronal degeneration in the corpus striatum. Lesions with excitotoxic features can also be produced by iodoacetate (81). This drug is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This indicates that similar excitotoxic type lesions with a similar brain distribution of pathology can be produced by inhibition of metabolism at different key points. By contrast cyanide, which is a complex IV mitochondrial inhibitor, has been reported to cause a different pattern of pathology, with damage to the globus pallidus, putamen, substantia nigra, subthalamic nucleus, and cerebellum in patients studied with brain imaging (100, 124). In general, all of these metabolic inhibitors tend to have a subcortical and cerebellar pattern of pathology rather than a cortical and hippocampal pattern of pathology. This is similar to the distribution of pathology in the triplet repeat diseases.

Huntingtin and Atrophin-1

The HD gene transcript encodes a protein (huntingtin) of 3144 amino acids with predicted molecular mass of 348 kDa. This protein has no strong homology to any other known proteins including the proteins encoded by the other genes with CAG repeat expansions (102). Huntingtin has no hydrophic domains suggestive of membrane spanning regions. It does have a putative repeating unit termed the "HEAT repeat" with no known function. These repeats appear in a number of other cytoplasmic proteins and are perhaps consistent with structural roles for the proteins in cells (6).

Antibodies raised against peptides corresponding to the predicted 17-amino-acid portion of huntingtin on the N-terminal side of the glutamine repeat, as well as anti-

bodies directed against other regions of the protein, react specifically on Western blots with a 350-kDa protein, showing that the CAG repeat is indeed translated (7, 33, 45, 53, 63, 93, 109, 122, 123). These antibodies indicated that the huntingtin protein, like the mRNA, is expressed widely throughout the body and in the brain (5, 70, 72, 118).

Immunocytochemical experiments using well-characterized antisera indicate that, in the brain, huntingtin is expressed widely, primarily in neurons, in perikarya, dendrites, axons, and neuronal terminals. In these studies, huntingtin was not observed in nuclei or mitochondria at either the light or the electron microscopic levels (33, 45, 109, 122). Moreover, transfected COS1 cells expressing the mouse huntingtin homologue with an expanded glutamine repeat derived from an HD patient appear to have the same distribution, with a lack of nuclear staining, as cells expressing the normal-length protein (122). The results of these studies appear to be in general agreement, although they differ in the relative levels of staining intensity in neuronal perikarya compared to the staining in dendrites and punctate neuropil. Huntingtin appears to be expressed in neurons at all stages of embryonic and postnatal development (16).

The immunohistochemical studies were supported by cell fractionation experiments showing that huntingtin is depleted in fractions enriched in cellular nuclei from rat brain (109) as well as in nuclear fractions prepared from lymphoblastoid cell lines (93). The huntingtin protein was also depleted from a fraction enriched in mitochondria (109). Thus, contrary to initial speculation, huntingtin does not appear to have a direct role in either cell nuclei or mitochondria, although some effect of expanded huntingtin on transcription factors or mitochondrial energy production cannot be ruled out. In some species huntingtin appears to have a more cytoplasmic or more particulate localization than in others (132). The reasons for this difference are uncertain.

High-resolution immunocytochemistry using colloidal gold-labeled secondary antibodies to detect immunoreactivity at the electron microscopic level has now been reported. DiFiglia et al. (33) showed the presence of label around synaptic vesicles in presynaptic nerve terminals as well as in the cytoplasm of postsynaptic dendrites. Gutekunst et al. (45) showed that, in Purkinje cell dendrites, some label appeared to be associated with microtubules, although label also appeared to be unassociated with any structure.

These data, together with the data on distribution and conservation of the protein, suggests that huntingtin is involved in important functions in a variety of cell types, perhaps a so-called "housekeeping" function. A possible role is the trafficking of vesicles within cells, including at least some synaptic vesicles in neurons, and such a role might include interactions with the cytoskeleton. Gutekunst et al. (45) suggested a role in the anchoring of vesicles or organelles to microtubules. Other possible functions, such as a role in signal transduction, have not been ruled out.

Is protein expressed from the expanded HD gene transcript, and, if so, how might its properties differ from those of the normal protein? Several groups have reported the detection of protein from the expanded allele (7, 45, 53, 63, 93, 94, 104, 109, 122, 123) by resolving the product of the expanded allele from the normal protein by electrophoresis on SDS polyacrylamide gels made with either a low percentage of total acrylamide or crosslinker. The separation appears to be greater than would be expected from a simple increase in molecular weight, suggesting that the expanded polyglutamine may alter the structure of the protein to cause it to run anomalously in SDS-polyacrylamide gel electrophoresis.

Several lines of evidence suggest that expansion of the polyglutamine tract does not markedly alter the subcellular distribution of huntingtin (93, 94, 122, 132). Careful experiments on the subcellular localization of huntingtin in both normal and patient tissue have recently been carried out using immunofluorescence (103, 126). In these experiments the subcellular localization of huntingtin appears to be enriched in the Golgi apparatus and the trans Golgi Network. Huntingtin co-localized with the transferrin receptor and with coated vesicles in peripheral cytoplasm. There was no striking difference in this localization in control lymphoblasts and lymphoblasts from a patient homozygous for the CAG expansion, strongly suggesting that, at least in these peripheral cells, the mutation does not dramatically alter the subcellular localization of the protein. Electron microscopic observations suggest that mutant huntingtin may accumulate in an endosomal/ lysosomal compartment in neurons in patients with HD.

Less is known about the DRPLA gene product (atrophin-1). Like the HD gene, the DRPLA gene open reading frame contains no significant homology to other known genes except for simple sequence repeats (80, 86, 90). However, atrophin-1, unlike huntingtin (so far), does appear to be a member of a gene family with at least one other member. This other gene, termed atrophin related protein or ARP1, has homology in both the N-terminus and C-terminus, but does not have a glutamine repeat (66). Unfortunately, the function of this

gene product is also unknown.

The expression of the DRPLA gene and its protein product are also widespread with a generally cytoplasmic appearance in neurons (75, 80, 85, 90, 105, 133). Like huntingtin, the atrophin-1 protein with the expanded repeat can be resolved from the normal protein by polyacrylamide gel electrophoresis (133).

Glutamine Repeats

The functions of glutamine repeats in proteins, if any, are unknown but may involve protein-protein interactions. Glutamine rich regions have been described in the factor interaction domain of transcription factors, although it is not clear whether glutamine repeats function like glutamine-rich regions. In the androgen receptor, for instance, the expanded glutamine repeat is located in a region thought to be important for interactions with other cell type transcription factors (1). In an assay of transcriptional activity, the length of glutamine repeats influences transcription, presumably via interactions with other transcription factors (37). Thus, glutamine repeats may influence protein-protein interactions.

Several hypotheses regarding such protein-protein interactions have recently been proposed. Green (42) suggested that proteins with long glutamine repeats might become substrates for transglutaminase activity, resulting in cross-linked products involving an _-_ glutamyl lysine isopeptide. The cross-linked protein could be degraded by proteolysis, but, presumably, the isodipeptide could not be degraded and may potentially have toxic effects within cells.

Another possibility involves noncovalent protein interactions. Perutz and co-workers (95, 96, 117) have proposed that glutamine repeats can form a "polar zipper," involving a β-sheet with hydrogen bonds between the main peptide chain and the side chain amides, either between two proteins with glutamine repeats or by self-association within one glutamine repeat-containing protein. Lengthening of the glutamine repeat would presumably increase the stability of the association.

How the preceding interactions might lead to toxicity is unclear. One model for toxicity suggests that glutamine repeats can precipitate within cells as insoluble β-pleated sheets (55). This hypothesis was based on the appearance of an apparent aggregate of a truncated glutamine-repeat-containing peptide fragment in gels. This model is analogous to the mechanism proposed for prion protein diseases.

Protein-Protein Interactions

The models described above do not explain the cell type-specific vulnerability of neurons in HD, DRPLA, and the other diseases. One possible explanation might arise if the glutamine repeat-containing protein interacted with other proteins that have a more restricted pattern of expression. Many groups have begun to search for such associated proteins.

Consistent with the possibility that huntingtin associates with other proteins is the recent demonstration that huntingtin appears to run in a gel filtration column with a broad peak of >1000 kDa. One protein with which huntingtin appears to interact indirectly (but not directly) is calmodulin (8). It may well interact with several proteins.

Several candidate interactor proteins for huntingtin have been identified. One protein is the enzyme (GAPDH) (21, 112). This interaction was initially demonstrated using a polyglutamine affinity column. Both huntingtin and atrophin-1 appeared to interact specifically with GAPDH. It was suggested in this initial paper that the interaction was dependent on the length of the glutamine repeat. However, it is not clear that this dependence is the case in vivo. A recent study has shown that GAPDH also interacts with ataxin 1 (the protein product of the SCA1 gene) and the androgen receptor (the protein product of the SBMA gene). However, these interactions do not appear to be altered by the length of the glutamine repeats (68). It was suggested that the huntingtin GAPDH interaction might inhibit GAPDH enzyme activity (101). However, data for this have not yet been presented. Nevertheless, this interaction is provocative in that GAPDH has recently been implicated in cell death (58). It is also a very striking finding in view of the recent demonstration that inhibition of GAPDH can itself cause striatal excitotoxic cell death as discussed above (81). It is conceivable that the interaction between glutamine repeat containing proteins and GAPDH could be involved in some form of metabolic toxicity in these diseases.

Huntingtin interacts with an E2 ubiquitin conjugating (hE2-25k) enzyme (64). This study also indicated that huntingtin is ubiquitinated, though neither the ubiquitinization or the interaction appeared to be dependent on the length of the glutamine repeat. Antibodies to the E2 enzyme cross reacted with a band which appeared to be selectively expressed in brain regions affected in HD, suggesting that this still unidentified protein could be involved in the selective pathology of HD. However, data supporting this possibility require further investigation.

Another proposal for pathogenesis caused by huntingtin involves proteolysis. A very dramatic recent find-

ing (38, 99) is that huntingtin can be cleaved by apopain (Caspase 3). Since the Caspase family of proteases has been implicated in apoptosis in many different cell systems, this is a very provocative finding. However, it is still unclear whether this is a cause or effect of cell death in HD.

Huntingtin also interacts with a protein identified using the yeast two-hybrid system, termed huntingtin interactor protein 1 (HIP1) (65, 128). This protein HIP1 is homologous to a previously identified yeast gene product called Sla2p (48). Mutations in this yeast gene interfere with endocytosis. HIP1 is also homologous to a portion of the talin molecule (97) which is involved in membrane cytoskeleton interactions. In the study by Kalchman et al. (65) the interaction between huntingtin and HIP1 was shown to be dependent on the length of the glutamine repeat. There was a striking decrease in the strength of the interaction when the glutamine repeat in huntingtin was expanded. This is consistent with the possibility that this interaction plays a role in the pathogenesis of HD. In addition, the huntingtin HIP1 interaction was restricted to the brain which would be consistent with the brain specific pathology of HD. How it might be relevant to the regional pathology in HD however still remains unclear. Biochemical evidence suggested that HIP1 is associated with the cytoskeleton consistent with its sequence homology to other cytoskeletal associated proteins. Thus, these interactions are consistent with the hypothesis that huntingtin is involved in some way in vesicle trafficking, especially endocytosis, and interaction with the cytoskeleton.

Another candidate protein was identified using the yeast two-hybrid system (73) using the N-terminus of the HD protein with an expanded glutamine repeat as the target. This protein, termed "huntingtin-associated protein-1" (HAP-1), interacted more strongly with huntingtin when huntingtin had an expanded glutamine repeat. Unlike the HD protein, the expression of HAP-1 is restricted to the brain; thus, HAP-1 could be implicated in the brain-specific pathology of HD. However, HAP-1 is not expressed selectively in vulnerable neurons. Moreover, it is not enriched in the striatum. Studies using in situ hybridization (74) have indicated that HAP-1 has an unusual pattern of expression similar to that of neuronal NOS, though more widespread. However, attempts to find a direct association between HAP-1 and neuronal NOS have been unsuccessful. HAP-1 does not interact with atrophin-1. Unfortunately, like huntingtin, HAP-1 has no strong homology to other proteins.

Recent experiments have used the yeast two-hybrid system to find proteins with which HAP-1 interacts. HAP-1 appears to dimerize perhaps due to coiled-coil motifs. In addition, the N-terminus of HAP-1 associates with a protein we have termed "Duo" because of its homology to a recently reported protein called "Trio" (32). Duo, like Trio, contains spectrin-like repeats. The cDNA coding for human Duo is the human homologue of a rat cDNA called P-CIP-10, identified because of its binding to the C-terminal domain of the integral membrane protein Peptidylglycine α-Amidating Monooxygenase (PAM) found in secretory and endocytic vesicles (2). Duo also contains a Rac1 GEF domain and a plecstrin homology (PH) domain with over 98% amino acid identity to the Rac1 GEF domain of Trio which has been demonstrated to be functionally active (32).

These data are suggestive of a role for Duo in regulating Ras-like signal transduction and thus raise the possibility that huntingtin may be indirectly involved in this activity as well. This is of special interest since Rac1 signal transduction is known to alter cell morphology including neuritogenesis (69,78). This could correspond to the morphologic alterations previously described in HD tissue (40,41). In addition, Rac1 has recently been implicated in activation of NADPH oxidase leading to the generation of superoxide radicals (57). If this pathway is active in neurons, it could potentially provide an indirect means by which huntingtin could activate free radical production in neurons. This possibility remains highly speculative at present however.

Preliminary experiments have recently identified two putative interactor proteins for atrophin-1. Both have WW domains (17) which may interact with PY motifs (XPPXY) in atrophin-1. Expression of one of these proteins appears to be restricted to the brain, while the other is expressed in all tissues examined. The relevance of these interactions to the pathogenesis of the disease is still uncertain. The strength of the interaction does not appear to be dramatically dependent on the length of the glutamine repeat in atrophin-1. Neither of these proteins interacts with huntingtin. One of them is homologous to members of a family of E3 ubiquitin-protein ligases (51), including the yeast Rsp5 protein. Strikingly, rsp5 mutants have a very similar phenotype to sla2 mutants in that both strains are deficient in the endocytosis and degradation of the Fur4 uracil permease (47). This suggests a possible link between huntingtin, atrophin-1 and endocytosis.

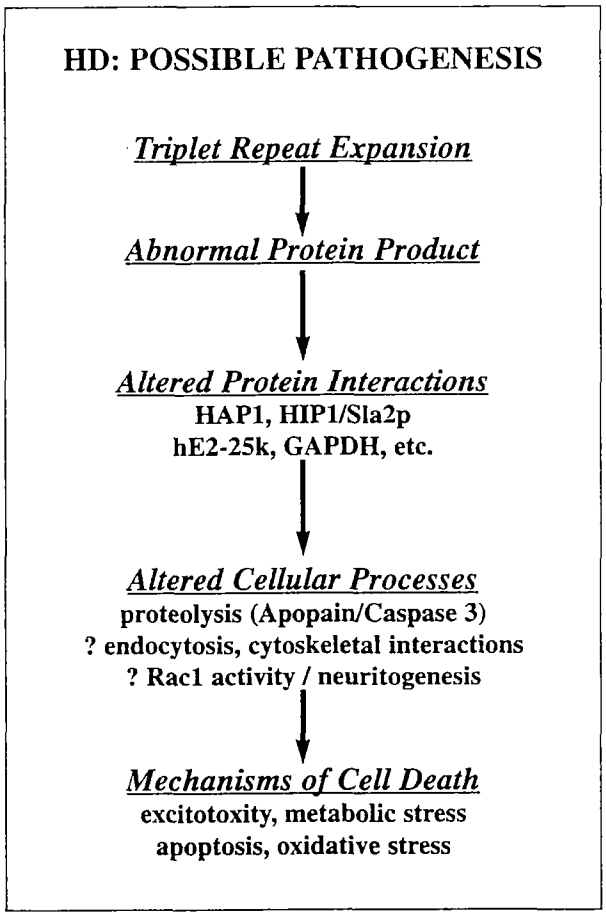

Figure 3. Hypothesized sequence of events in the pathogenesis of HD. This scheme is based on our present knowledge and will no doubt be modified by future evidence. See text for details.

Conclusions

Two themes seem to be emerging which may be relevant to understanding the regional pathology of HD, DRPLA, and the other glutamine repeat diseases. The first is the concept of intrinsic cellular vulnerability to metabolic poisons. Different metabolic poisons give rise to slightly different patterns of pathology, which bear some similarities to the different glutamine repeat diseases. However, how the presence of the expanded glutamine repeat in these protein products might lead to metabolic vulnerability is still uncertain. As discussed above, the proposal that the glutamine repeat expansions by themselves are sufficient to cause pathology appears over-simplified. The properties of the disease gene protein products must also be critical.

The other theme is that of specific protein interactions. It is emerging that huntingtin and atrophin-1 each interact with a number of cellular proteins. At least some of these interactions appear to be dependent on the length of the glutamine repeat and thus, could be implicated in pathogenesis of the illness. The emerging evidence that huntingtin is located in proximity to intracellular vesicles (possibly endosomes) and cytoskeletal elements is certainly consistent with the identification of interacting proteins which are also localized to endosomes and cytoskeleton. An outline of possible pathogenesis based on current data is shown in Figure 3.

It should be kept in mind that the gain of function pathogenic process of the glutamine repeat diseases may not be closely related to the normal function of these proteins. Nevertheless, the location of the proteins within cells is likely to be relevant. It does appear that huntingtin and atrophin-1 interact with different proteins. Atrophin-1 does not interact with HAP1 or huntingtin. Huntingtin does not appear to interact with the two newly identified atrophin-1 interactors. Therefore, these protein interactions may help explain the differences in cellular pathology of the different diseases. Future work with cell models and animal models may help clarify the relationship of this emerging biochemistry to the pathogenesis of these diseases.

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Note added in proof: the data descibing Duo are now in press (136). In addition, the full length rat homologue is now published with additional functional information (137) and now termed "Kalirin."

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