Huntington's disease: from gene to potential therapy

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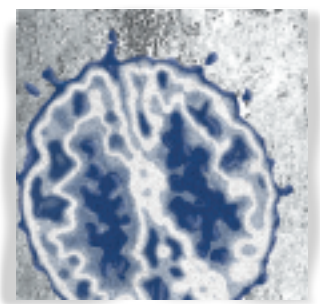

Huntington's disease (HD) is a progressive, late-onset neurodegenerative illness with autosomal dominant inheritance that affects one in 10 000 individuals in Western Europe. The disease is caused by a polyglutamine repeat expansion located in the N-terminal region of the huntingtin protein. The mutation is likely to act by a gain of function, but the molecular mechanisms by which it leads to neuronal dysfunction and cell death are not yet known. The normal function of huntingtin in cell metabolism is also unclear. There is no therapy for HD. Research on HD should help elucidate the pathogenetic mechanism of this illness in order to develop successful treatments to prevent or slow down symptoms. This article presents new results in HD research focusing on in vivo and in vitro model systems, potential molecular mechanisms of HD, and the development of therapeutic strategies.

A growing number of neurodegenerative disorders have been found to belong to the group of CAG triplet repeat disorders, including Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), dentatorubral palidoluysian atrophy, Machado-Joseph disease/spinocerebellar ataxia type 3, and spinocerebellar ataxias types 1, 2, 6, and 7.[1] All these illnesses are caused by an elongated CAG repeat located in the coding region of the respective genes, which is translated into a polyglutamine tract. The mechanism by which CAG repeats elongate is currently unknown and is the subject of intensive investigation.[2]

Characteristic features of CAG repeat disorders are autosomal dominant inheritance (except SBMA), late onset, selective neurodegeneration, genetic anticipation, a pathological threshold at which the mutation becomes virulent, and an inverse correlation between CAG repeat length and age at disease onset. The number of glutamines in the normal (<35 residues) and abnormal ranges (>35 residues) are similar in each disease protein, with the exception of spinocerebellar ataxia type 6 (SCA6). The disease proteins show no homology with each other except the glutamine repeat, suggesting that the elongated glutamine tract confers a toxic gain of function to each disease protein. The current body of evidence supports the hypothesis that expanded polyglutamine repeats undergo a conformational change leading to abnormal protein–protein interactions, multimerization, and the formation of insoluble protein aggregates.[3-5] Indeed, abnormal neuronal inclusions have been detected in the brains of patients.[6,7] Although the causal relationship between aggregate formation and disease remains to be proven, the gradual deposition of disease protein in neurons is consistent with the late onset and progressive nature of symptoms. Furthermore, the process of aggregate formation is ultimately associated with degeneration of mammalian cells.[8] Analysis of in vitro and in vivo model systems support the hypothesis that glutamine repeat disorders, like Alzheimer's disease and Parkinson's disease, are caused by an aggregation-based pathogenetic mechanism. However, there are also studies that suggest that the process of aggregate formation may even be beneficial to neu-

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Selected abbreviations and acronyms

HAP1	*huntingtin-associated protein–1*
HD	*Huntington's disease*
HIP1	*huntingtin interacting protein–1*
NII	*neuronal intranuclear inclusion*
SBMA	*spinal and bulbar muscular atrophy*

ronal cells.[9,10] Aggregation as the main detrimental factor in neurodegeneration in HD and related illnesses has been a debated issue in recent years.

This article will describe recent advances in understanding the pathogenesis of HD, the most common and most studied of the glutamine repeat disorders. Different model systems for the screening and analysis of potential therapeutic molecules have been established and have yielded exciting results with regard to halting the formation of insoluble protein aggregates.

Clinical features and neuropathology of Huntington's disease

HD begins gradually with mood disturbances, increasing involuntary movements (chorea), and cognitive impairment, finally leading to dystonia and severe dementia. The first symptoms typically appear in mid-life (late fourth and fifth decade); however, there are also juvenile and late-onset cases. Within 15 to 20 years after its onset, the disease inexorably progresses to death. Mood abnormalities often start appearing a few years before movement dysfunction, which comprises both involuntary as well as impaired voluntary movements. Chorea is observed in ~90% of all HD patients and increases during the first 10 years of the illness, while dystonia is infrequent in the early symptomatic period but becomes prominent at the late stages of the illness. Cognitive disturbances begin with a loss of mental flexibility and progress to profound dementia.

The clinical progression of HD is associated with degeneration of the striatum. HD is classified into five pathological grades, ranging from microscopically undetectable abnormalities of patient brains to extensive atrophy.[11] All grades exhibit a loss of brain weight (up to 30%) resulting from neuronal cell death. The extent of neuronal cell death is directly related to the severity of disease. In the basal ganglia, the caudate nucleus is more severely affected than the putamen or the globus pallidus. The specific progressive atrophy in these brain regions is associated with reactive astrocytosis.[12] Within the striatum there is selective loss of medium spiny GABA (γ-aminobutyric acid)–ergic neurons, which project into the pallidum forming the indirect striatopallidal pathway. Prior to cell death, neuronal dysfunction is manifested by abnormalities of dendritic endings. In addition to atrophy in the striatum, extensive neuronal cell loss also occurs in the deep layers of the cerebral cortex, white matter, hippocampus, amygdala, and thalamus.[13]

The disease gene and its protein

The human HD gene is located in the chromosomal region 4p16.3 and was isolated by positional cloning approaches.[14] It contains 67 exons and encodes the huntingtin protein of 3144 residues with a molecular mass of about 350 kd. The mutation underlying HD is an unstable CAG trinucleotide repeat expansion in the first exon of the gene. It ranges from 6 to 37 units in healthy individuals, and 38 to 180 units in HD patients.[15,16] The CAG repeat is translated into a polyglutamine stretch, which is conserved in vertebrates, containing 7 glutamines in the mouse[17] and only 4 in the puffer fish,[18] but is absent from the *Drosophila* protein.[19] The predicted huntingtin protein sequence is highly conserved between human, mouse, and puffer fish, but shows no significant homology with other proteins in databases. The only functional motives that have been discovered are a putative leucine zipper and a HEAT repeat.[20] HEAT repeats consist of two hydrophobic α-helices and were found in proteins involved in cellular transport processes. We have found that the huntingtin interacting protein–1 (HIP1) associates with the HEAT repeat.[21] However, whether this sequence motive is essential for protein–protein interaction remains to be determined. HIP1 has been identified using the yeast two-hybrid system. The predicted amino acid sequence of HIP1 exhibits significant similarity to cytoskeleton proteins, suggesting that HIP1 and the huntingtin protein play a functional role in the cell filament networks and/or vesicle trafficking. For example, HIP1 is homologous to the yeast protein Sla2p,[22] which associates with the membrane cytoskeleton and plays a functional role in endocytosis.[23] Recently, colocalization of HIP1 and huntingtin

with clathrin-coated vesicles in mammalian cells has been described, suggesting that both proteins also play a functional role in endocytosis in higher eukaryotes.[24,25] This hypothesis is substantiated by the finding that huntingtin and its associated protein, huntingtin-associated protein–1 (HAP1),[26] are transported along microtubules in axons.[27] Furthermore, direct binding of HAP1 with p150Glued of the dynactin complex, which is critical for retrograde movement of vesicles along microtubules, has been described.[28] Together these findings indicate that a protein complex consisting of the proteins HIP1, HAP1, and huntingtin is functionally involved in endocytosis and retrograde transport of clathrin-coated vesicles along microtubules. However, additional cell biology and biochemical studies will be necessary to address this hypothesis in more detail.

Using the yeast two-hybrid system we have also demonstrated that the SH3-containing Grb2-like protein SH3GL3 associates with huntingtin.[29] This protein is preferentially expressed in brain and testis and selectively interacts with the proline-rich region in huntingtin, which is located immediately downstream of the polyglutamine tract. The SH3GL3 protein, as well as its homologous proteins SH3GL1 and SH3GL2, belongs to a novel SH3-containing protein family. Members of this family contain the SH3 domain at the C-terminus that is evolutionarily conserved and drives protein–protein interactions through proline-rich ligands.[30] In the central nervous system, these proteins play a major role in the signal transduction from membrane receptors and the regulation of the exocytic/endocytic cycle of synaptic vesicles.[31] Thus, enhanced binding of SH3GL3 to huntingtin with a polyglutamine sequence in the pathological range (eg, 50 glutamines) could result in dysregulation of the endocytic/exocytic cycle in mammalian cells.

In order to address the functional role of huntingtin, HIP1, and SH3GL3 in synaptic vesicle transport in more detail, the homologous mouse genes were mapped and cloned.[32-34] The generation of HIP1 and SH3GL3 knockout as well as transgenic animal models will help elucidate the normal function of huntingtin and may also help to understand the key steps in the pathogenesis of HD.

Neuronal inclusions and neuropathology

In order to study the effect of an elongated polyglutamine sequence on neuronal dysfunction and neurodegeneration in vivo, Mangiarini et al[35] generated the first HD transgenic mice. In these animals, exon 1 of the human HD gene carrying a CAG repeat of 115 to 156 units was expressed under the control of the HD promoter. Strikingly, expression of the mutant huntingtin fragment resulted in the development of a progressive neurological phenotype very similar to HD, including tremor, epileptic seizures, involuntary movements, and cell loss. This indicates that expression of a truncated huntingtin fragment with a polyglutamine sequence in the pathological range is sufficient for the development of a neurological phenotype with characteristic features of HD.

Davies et al[36] observed that these transgenic animals developed pronounced neuronal intranuclear inclusions (NIIs) containing huntingtin and ubiquitin prior to the development of the neurological phenotype, indicating that formation of NIIs is a prerequisite for the development of neuronal dysfunction in HD.

Within the last few years, several other laboratories have confirmed the appearance of NIIs using different transgenic mouse models of HD.[37-39] Full-length huntingtin protein as well as truncated versions of the protein with a polyglutamine sequence in the pathological range (40-150 glutamines) were expressed in mice under the control of different promoters. The majority of these studies suggest that the formation of NIIs is correlated with the appearance of progressive neuronal dysfunction and toxicity. Thus, it is reasonable to assume that reduction of inclusion body formation and huntingtin aggregation may have a beneficial effect on disease progression in HD patients. Using a conditional mouse model of HD, Yamamoto et al[40] demonstrated that blocking the expression of mutant huntingtin protein in neurons resulted in the disappearance of inclusions and the behavioral phenotype. Therefore, reduction of HD protein expression in patients and/or stimulation of natural clearance mechanisms could be effective therapeutic strategies for HD.

Apart from NIIs, inclusion bodies with aggregated huntingtin protein were recently detected in axons and axon terminals of striatal neurons.[41] These structures were termed neuropil aggregates. The formation of these aggregates is likely to affect specific neuronal functions such as axonal transport and neurotransmitter release or uptake in axon terminals. Therefore, the deposition of mutant huntingtin protein in the terminals of striatal

neurons, which are affected most in HD, may contribute to the selective neuropathology of HD.

After the discovery of NIIs in brains of transgenic animals,[35] similar structures were detected in postmortem brains of HD patients.[6,7] NIIs were found in neurons but not in glia cells. Immunohistochemical studies showed that they are most abundant in the striatum and the cerebral cortex, the areas most affected by HD. In the striatum, inclusions were found in the medium spiny neurons that are selectively lost during HD. NIIs in patient brains are detected by antibodies directed against the N-terminus of huntingtin, but not by antibodies that recognize the C-terminus of the protein, indicating that a truncated N-terminal huntingtin fragment rather than the full-length protein is present in the NIIs of patients. Like the NIIs in transgenic animals, the NIIs in patients were stained with anti-ubiquitin antibodies. These results suggest that the truncated huntingtin protein present in the inclusion bodies is ubiquitinated but cannot be degraded by the proteasome system.[42] Ultrastructural studies revealed that NIIs in patient brains contain aggregated huntingtin protein with a fibrillar and granular morphology. In addition, dystrophic neurites containing aggregated huntingtin protein were detected.[6] Dystrophic neurites are known to result from dysfunction of neuronal retrograde transport. Formation of insoluble huntingtin aggregates could alter this process in neuronal cells.

It remains unclear to date whether the formation of NIIs, dystrophic neurites, or neuropil aggregates is the cause or merely a consequence of neurodegenerative disorders. Using neuronal cell culture model systems and transgenic animals, Saudou et al[9] and Klement et al[10] presented evidence that the formation of inclusion bodies could be nontoxic or even beneficial to neuronal cells. They showed that overexpression of disease proteins with a polyglutamine sequence in the pathological range is toxic for neuronal cells, but inclusion formation does not contribute to toxicity. However, using cell culture as well as transgenic animal model systems in the presence of more physiological amounts of mutant huntingtin, cell death was observed only after fibrillar structures had formed. We therefore propose that formation of aggregates and subsequently of inclusion bodies is a key step in the development of late-onset progressive neurodegenerative disorders.

Huntingtin protein aggregation and therapeutic strategies

If aggregation is crucial, preventing aggregation must slow down disease progression. We have developed a number of in vitro and in vivo strategies to address this issue, the creation of a drug screen assay being one of them.

Formation of insoluble huntingtin protein aggregates was reproduced in vitro. We found that HD exon 1 protein fragments with polyglutamine tracts in the pathological range (>37 glutamines), but not with a polyglutamine tract in the normal range (20-32 glutamines), form high-molecular-weight protein aggregates.[5,43] Electron micrographs of these aggregates revealed a characteristic fibrillar or ribbon-like morphology, reminiscent of scrapie prion rods and the β-amyloid fibrils found in Alzheimer's disease.[5] The fibrillar structures are thought to result from the polyglutamine sequences acting as "polar zippers." Perutz[3] proposed that expansion of polyglutamine repeats beyond a critical length of 41 glutamines may lead to a phase change from random coils to hydrogen-bonded hairpins that self-assemble into insoluble protein aggregates. Our in vitro experiments with glutathione *S*-transferase (GST)–HD exon 1 fusion proteins support this hypothesis, suggesting that the structural transition caused by expansion and required for aggregate formation occurs between 32 to 37 glutamines. Polyglutamine tracts with 37 or more glutamines readily self-assemble into insoluble protein aggregates, whereas polyglutamine tracts with less than 32 glutamines did not show any evidence of fibril formation. Interestingly, it has been shown that the pathological range of the polyglutamine sequence in HD is between 38 to 41 glutamines, with no HD case reported with fewer than 38 glutamines, nor any individual with more than 41 glutamines having remained unafflicted by HD. The threshold for the formation of insoluble huntingtin fibrils in vitro is remarkably similar to the pathological threshold in HD.

Jarrett and Lansbury[44] proposed that the self-assembly of β-amyloids into fibrillar structures in Alzheimer's disease occurs by a nucleation-dependent mechanism. We found that the formation of amyloid-like huntingtin fibrils in vitro and in vivo critically depends on polyglutamine repeat length, protein concentration, and time. Furthermore, huntingtin aggregation can be seeded by

preformed fibrils, suggesting that fibrillogenesis in HD, as in Alzheimer's disease, is caused by nucleation-dependent polymerization.[43] Our findings that the assembly of huntingtin aggregates requires the formation of a nucleus and is time- and protein concentration–dependent may account for the late onset and progressive phenotype in HD. Although fibril formation occurs within hours in the in vitro system, it may take years in neuronal cells of HD patients. The concentration of mutant huntingtin in medium spiny neurons, which are affected most in HD, is unknown, but it is likely to be much lower than that used in the in vitro aggregation assays. Thus, we suggest that the lag time during which huntingtin dimers, trimers, and oligomers are formed in vivo is significantly elongated in HD patients.

For the identification of huntingtin aggregation inhibitors, we have developed a rapid and sensitive filter retardation assay, which is suitable for the high-throughput screening of drugs that prevent aggregate formation.[45] This assay is based on the finding that HD exon 1 aggregates are insoluble in sodium dodecyl sulfate (SDS) and are retained on a cellulose acetate filter, whereas monomeric forms of the HD exon 1 protein do not bind to this filter membrane. The captured aggregates are then detected by simple immunoblot analysis using a specific anti-huntingtin antibody. Using the filter retardation assay, we first tested a number of known inhibitors of β-amyloid, PrP^{scr}, and microtubule fibril formation for their effect on huntingtin aggregation in vitro.[46] We found that Congo red, thioflavine S, chrysamine G, and Direct fast yellow inhibited HD exon 1 protein aggregation in a dose-dependent manner, whereas other potential inhibitors of β-amyloid formation, such as thioflavine T, gossypol, melatonin, and rifampicin, had little or no effect on huntingtin aggregation. The results obtained in vitro were confirmed in cell culture model systems. Furthermore, we found that the monoclonal antibody 1C2, which specifically recognizes the elongated polyglutamine stretch in huntingtin, and the heat shock proteins Hsp70 and Hsp40 are potent inhibitors of huntingtin aggregation.[46,47] Interestingly, the addition of heat shock proteins to in vitro aggregation reactions shifts the self-association pathway of huntingtin from fibrillar to amorphous aggregation. This suggests that in vivo chaperones may have the potential to transform toxic fibrillar aggregates into nontoxic aggregates, which can then be degraded by the ubiquitin/proteasome system. Thus, small molecules that activate a heat shock response in neurons may be effective in delaying the onset and progression of HD. However, additional research using in vitro and in vivo model systems will be required to show whether an increase in chaperone levels in neurons is a viable therapeutic strategy.

Conclusions

Inhibition of huntingtin fibrillogenesis by small molecules is a very attractive therapeutic strategy. Drugs that bind to the mutant huntingtin protein should delay the onset and progression of HD. The future challenge will be to find small chemical compounds that have reasonable brain permeability, and per se are nontoxic to neuronal cells. In order to find such compounds, we have performed high-throughput screening using an automated filter retardation assay. Within the last year we have tested more then 180 000 different chemical compounds and identified about 700 small molecules that prevent huntingtin aggregation in vitro. These compounds are currently being tested in cell culture model systems of HD. We were able to reduce aggregate formation in mammalian cells; as a consequence, cytotoxicity was lowered. This is a very important finding, because it shows for the first time that there is a direct link between the process of aggregate formation and disease. The next challenge will be to test these substances in transgenic animals for their ability to cross the blood–brain barrier, to dissolve neuronal inclusions or prevent their formation, and to reduce neurodegenerative symptoms. If this proves successful, one could think of moving on to clinical trials. Since the identification of the gene for Huntington's chorea in 1993, this would represent a major milestone in HD research and also in molecular medicine generally, because for the first time a causal therapy for an inherited disease would be within reach. It would also have positive implications for functional genomics, because it would be the first time the strategy of finding a gene with a positional cloning approach and subsequent functional analysis and characterization of the pathogenetic mechanism had been able to lead to a causal therapy of an illness. ❑

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Enfermedad de Huntington: desde los genes a la potencial terapia

La Enfermedad de Huntington (EH) es una patología progresiva, neurodegenerativa, de aparición tardía con herencia autosómica dominante que afecta a uno en 10 000 individuos en Europa Occidental. La enfermedad es causada por una expansión repetida de poliglutamina ubicada en la región N-terminal de la proteína huntingtina. La mutación es probable que actúe por un aumento de la función, pero los mecanismos moleculares que conducen a la disfunción neuronal y la muerte celular aún no se conocen. La función normal de la huntingtina en el metabolismo celular tampoco está aclarada. No hay tratamiento para la EH. La investigación en la EH debe ayudar a esclarecer los mecanismos patogenéticos de esta enfermedad en orden a desarrollar tratamientos exitosos para prevenir o reducir los síntomas. Este artículo presenta nuevos resultados en la investigación de la EH focalizados en sistemas modelo en vivo e in vitro, potenciales mecanismos moleculares de la EH y el desarrollo de estrategias terapéuticas.

La maladie de Huntington : du gène au traitement potentiel

La maladie de Huntington (MH) est une pathologie neurodégénérative progressive d'apparition tardive à transmission dominante autosomique qui atteint un individu sur 10 000 en Europe de l'Ouest. Une amplification génique de la polyglutamine située sur la région N-terminale de la protéine huntingtin est à l'origine de la maladie. La mutation agit probablement par l'intermédiaire d'un gain fonctionnel mais les mécanismes moléculaires qui conduisent à la dysfonction neuronale et à la mort cellulaire ne sont pas encore connus. Le rôle normal de la protéine huntingtin dans le métabolisme cellulaire est également obscur. Il n'existe pas à l'heure actuelle de traitement pour la MH. La recherche devrait fournir des éclaircissements sur les mécanismes pathogéniques de la maladie et permettre le développement de thérapeutiques capables de prévenir ou de ralentir les symptômes. L'article qui suit présente les nouveaux résultats de la recherche dans la MH, tout particulièrement ceux concernant les systèmes de modélisation in vivo *et* in vitro*, les mécanismes moléculaires potentiels de la MH et le développement des stratégies thérapeutiques.*

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