

Huntingtin Protein and Protein Aggregation

What Causes the Onset of HD?

An Explanation of Terms:

*Please note that although "Huntington's disease" is spelled with an "o," the correct spelling of the **protein** involved is "huntingtin" with an "i." The scientific literature on HD refers to the **gene** as both the "Huntington gene" and the "huntingtin gene." For the purposes of this website, we will refer to the **gene** as the "Huntington gene" or the "HD gene."*

Current research shows that there is an abundance of information to be learned regarding the genetic origins of HD. Let's trace HD's beginnings from the molecular level, exploring the relationships between a gene, a protein, aggregation "clumps," neural cell death, and the disease itself.

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Huntingtin Protein and Protein Aggregation Part 1

What Causes the Onset of HD?

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The Huntington Gene

Recall that a [gene](#) is a section of DNA made up of four different nucleotide bases, abbreviated by the letters A, T, C, and G. The order of these bases determines the protein “product” of the gene. (To read more about DNA, click [here](#).) Everyone has a gene that codes for [huntingtin protein](#), a protein found in the cells of the body, which we will discuss later. Towards the beginning of this gene, the three-letter [codon](#) sequence C-A-G is repeated a few times. Each C-A-G sequence codes for the amino acid [glutamine](#), a protein building block. People with HD simply have an increased number of these C-A-G repeats toward the beginning of their Huntington gene, coding for an excess number of glutamines in their huntingtin protein. This glutamine extension in altered huntingtin leads to further problems, as we will see.

Huntingtin Protein and Protein Aggregation Part 2

What Causes the Onset of HD?

What is the *function* of huntingtin protein?

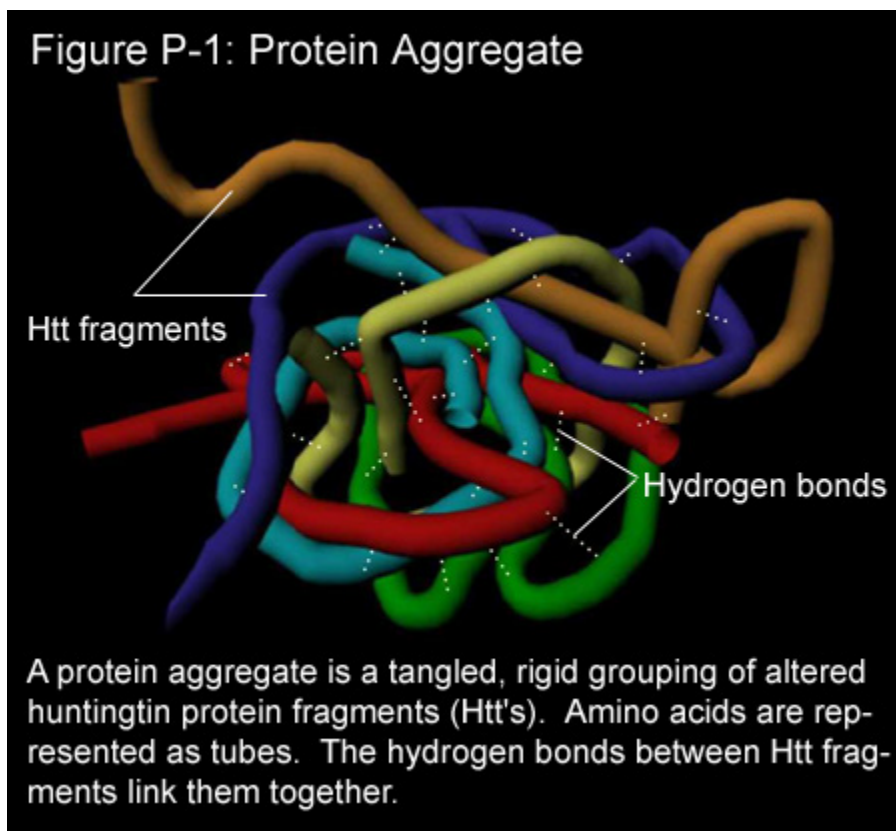
Although researchers are still investigating huntingtin protein’s exact purpose, it appears to play a critical role in [nerve cell](#) function. Huntingtin regularly interacts with proteins found only in the brain. Thus, altered huntingtin is most disruptive to nerve cells, even though it is found throughout the body.

Huntingtin Protein and Protein Aggregation Part 3

What Causes the Onset of HD?

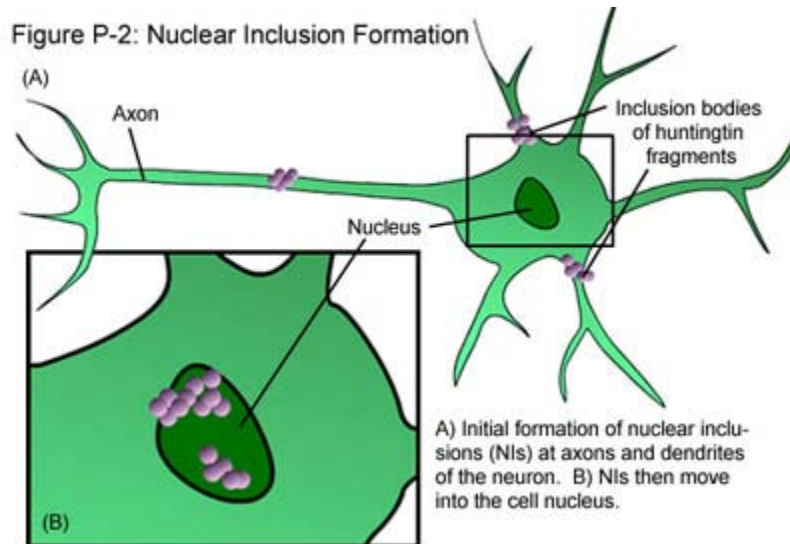
How can the *structure* of huntingtin be altered?

The altered form of huntingtin protein has been found in the autopsied brains of patients who have died of HD. Normal, functioning huntingtin protein contains 10-35 glutamines. (Click [here](#) to read more about glutamine expansion numbers.) In contrast, altered HD huntingtin protein (called “[Htt](#)” by researchers), contains 40 or more glutamine repeats, resulting from the genetic mutation discussed above. The extended number of glutamine repeats in Htt characterizes HD as one of nine [polyglutamine expansion disorders](#). (To read more about these disorders, click [here](#).)



Because glutamine is a polar, or “charged” molecule, the overabundance of glutamine causes links to form within and between proteins. Htt molecules “stick” to one another, forming strands that are held together by hydrogen bonds. Rather than folding into functional proteins, they develop into tangled, rigid groupings known as [protein aggregates](#). (See Figure P-1.) These fibrous protein aggregates accumulate and interfere with nerve cell function by entrapping key cell regulatory factors. Researchers are exploring whether protein aggregates are a cause or a consequence of neurodegenerative diseases such as HD. ***Are aggregates always harmful, causing nerve cell dysfunction and death, or are they***

simply part of a cellular defense mechanism? We will explore this question throughout this section.



In a process similar to the formation of aggregates, the excess glutamines in Htt can lead to a type of protein bundling known as [neuronal inclusions \(NI\)](#), or inclusion bodies. NIs initially form at the [axons](#) and [dendrites](#) of nerve cells in specific areas of the human brain, producing the damaged neurons characteristic of HD. Subsequently, an [enzyme](#) cuts Htt into smaller fragments which enter the nerve cell nuclei, forming more clumps at the [centrosomes](#). (See Figure P-2.)

Neuronal inclusions cause problems for the cell. They can cause significant changes in cell structure, “trap” and interfere with the normal production of other proteins, and ultimately become toxic to the nerve cell. Thus, the formation of NIs and the neurodegenerative symptoms of HD are clearly linked.

Huntingtin Protein and Protein Aggregation Part 4

What Causes the Onset of HD?

Does mutant huntingtin protein itself kill nerve cells?

Not directly. As we have seen, one way Htt indirectly leads to nerve cell damage and toxicity is through the formation of protein aggregates and [neuronal inclusions](#). These

structures can interfere with several crucial cellular proteins and systems. Researchers have explored a number of hypotheses regarding the mechanisms by which huntingtin aggregates cause cell dysfunction in HD.

Huntingtin Protein and Protein Aggregation Part 5

What Causes the Onset of HD?

Altered Huntingtin and CREB-binding Protein

Recent studies have shown that altered huntingtin can “kidnap” smaller proteins from their usual locations, preventing them from functioning normally within nerve cells. A recent Johns Hopkins University study showed that Htt entangles and inhibits [CREB-binding protein](#) (also known as CBP), a smaller regulatory protein that is key for cell survival. CBP has its own tract of 18 glutamines, and these glutamines interact directly with the expanded Htt glutamine chain. Huntingtin aggregates pull CBP away from its normal position alongside the DNA in the nucleus. Live mouse models with altered huntingtin and autopsied brains of patients who have died of HD show very reduced amounts of CBP, suggesting that it has been pulled away from the DNA and sequestered by the altered huntingtin.

Once seized, CBP is out of service. It can no longer accomplish its normal function of activating [transcription](#) or “turning on” genes for survival pathways. Fewer proteins are produced, ultimately leading to nerve cell death. However, researchers were able to fully halt and reverse this degenerative process in the laboratory. This reversal was accomplished by inserting an engineered form of CBP that did not have glutamine repeats. Since Htt interacts directly with the CBP’s glutamines, the modified CBP was not recognized by Htt. The modified CBP was not sequestered in huntingtin inclusion bodies, and the nerve cells survived.

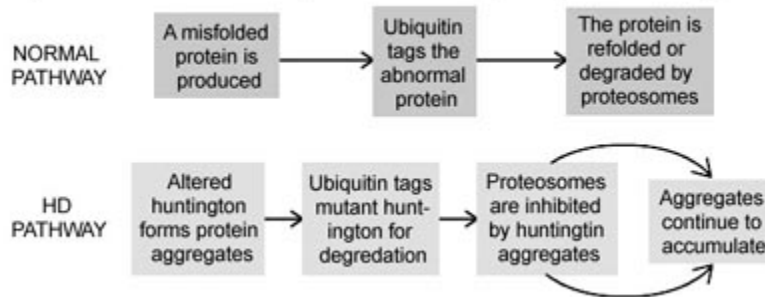
In addition, other compounds known as [histone-deacetylase inhibitors \(HDAC inhibitors\)](#) have been shown to compensate for the negative effects of CBP. It has been shown in fruit flies that HDAC inhibitors reduce the lethal effects of the altered huntingtin. These developments indicate potential targets for new drug treatments, but further research is still needed.

Huntingtin Protein and Protein Aggregation Part 6

What Causes the Onset of HD?

Altered Huntingtin and the Ubiquitin-Proteasome System

Figure P-3: Altered huntingtin and the ubiquitin proteasome system



Normal cells have a mechanism for quality control called the [ubiquitin-proteasome system \(UPS\)](#). The UPS is a protein-chaperone system which tags misfolded or damaged proteins for re-folding, or more commonly, for degradation. In contrast, protein aggregates resulting from the altered HD gene have a high molecular mass and are sequestered to the [aggresome](#) region of a cell. (See Figure P-3.)

Huntingtin Protein and Protein Aggregation Part 7

What Causes the Onset of HD?

Why is the altered form of huntingtin not broken down by the cell, as normally occurs with irregular proteins?

A recent Stanford University study suggests that protein aggregates impair UPS function, another explanation for how these huntingtin bundles can lead to the death of neurons. Large clumps of defective protein accumulate and scar neurons, which then cannot survive and reproduce. This conclusion about the relationship between protein aggregation, UPS function, and neurodegenerative disease was drawn from an experiment involving mutant cystic fibrosis protein and mutant huntingtin protein. Both of these proteins contain a polyglutamine repeat sequence, giving them the tendency to aggregate. Within human embryonic kidney cells, proteins were tagged with a special form of green fluorescent protein (GFP) which glows only when proteins remain intact. Throughout the experiment, mutant, aggregation-prone proteins continued to glow, showing that they were resistant to degradation, and thus, that the UPS system was

stalled. Fluorescence was higher in cells containing aggregates than in cells without, suggesting that protein aggregation led to the UPS disruption.

Huntingtin Protein and Protein Aggregation Part 8

What Causes the Onset of HD?

What is the process by which protein aggregates interfere with the UPS system?

Well, one clue is that levels of the normal protein [ubiquitin](#) are not diminished, and it continues to tag mutant huntingtin for degradation. Thus, it has been suggested that the abnormal proteins clog the other component of the UPS system, the proteasomes. Proteasomes are “master controllers” which normally break down proteins that have been tagged by ubiquitin. Protein aggregates may occupy the [proteosomes](#), inhibiting their normal activity.

Now let’s return to our earlier question: Are huntingtin aggregates a cause or consequence of HD? While the answer remains unclear, the Stanford study concludes that aggregates themselves can contribute to cell toxicity by impairing the UPS system. In a vicious, positive-feedback cycle, the aggregates inhibit the pathway that destroys them. Hence, they continue to build up, resulting in further impairment of UPS function. This accumulation of mutant proteins is gradual, which may explain the late onset of HD.

Future research in this area may focus on *how* and *why* the proteasomes shut down in the presence of protein aggregates. Answers to these questions may lead to treatments that can dissolve aggregate proteins or prevent them from forming, if it is proven that they are indeed only harmful.

Huntingtin Protein and Protein Aggregation Part 9

What Causes the Onset of HD?

Future Challenges for Huntingtin Research

Clearly, we have much to learn from exploring the relationships between the Huntington gene, huntingtin protein, protein aggregation, and neural cell death that characterize HD. The effects of HD huntingtin protein on the CBP and UPS pathways are examples of molecular-level problems leading to neural cell death. Future research in this area will likely continue to explore the following questions:

- What is the process by which the proteins aggregate?
- How can the cell get rid of these aggregates?
- What other protein-protein interactions are involved in this disease?

Answers to these questions may point to further possibilities for treatment of HD.

C. Barnard, 1-28-02

For further reading:

1. Bence NF, Sampat RM, Kopito RR. "Impairment of the ubiquitin-proteasome system by protein aggregation." Science 2001. 292: 1552-1555.
A fairly technical paper, reporting a study which shows that protein aggregation impairs the ubiquitin-proteasome system.
2. Helmuth, Laura. "Protein clumps hijack cell's clearance system." Science 2001. 292: 1467-1468.
A less technical, more reader-friendly summary of the paper listed above.
3. Kopito RR, Ron D. "Conformational disease." Nature Cell Biology. 2: 207-209.
A fairly technical overview of diseases caused by protein aggregation pathways.
4. Nucifora FC, Jr, et al. "Interference by huntingtin and atrophin-1 with CBP-mediated transcription leading to cellular toxicity." Science 2001. 291(5512): 2423-2428.
A highly technical paper regarding the negative effect produced when the altered huntingtin protein "kidnaps" a smaller protein called CBP.
5. Steffan JS, et al. "Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila." Nature 2001. 413: 739-743.
A fairly technical paper that discusses the interaction between altered huntingtin protein and CBP, as well as the effects of HDAC inhibitors.
6. Waelter S et al. "Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation." Molecular Biology of the Cell 2001. 12(5): 1393-1407.
A fairly technical paper regarding the relationship between inclusion bodies and the ubiquitin-proteasome system.