A Molecular Switch:

Studies of phosphorylation on the amino terminus of the huntingtin protein suggest a new direction for treatment

New research by Coalition for the Cure scientist Dr. Leslie Thompson, Dr. Joan Steffan, Dr. William Yang, and colleagues have identified posttranslational modifications of the HD protein which may lead to a major treatment for the disease.

Dr. Thompson, Dr. Steffan and colleagues have been studying the seventeen amino acids that precede the polyglutamine region (the CAG repeats) on the huntingtin protein. This area is called the N-Terminal 17 or NT17 domain. Cumulative research by Dr. Steffan and others has shown that this is a significant area for the disease process in Huntington's Disease since it is involved in subcellular localization, stability, cellular toxicity, and accelerating aggregation.

Recent research into ataxin-1, a different polyglutamine disease, has shown that phosphorylation in a region of the ataxin protein that is similar to the NT17 of huntingtin is a critical process in the disease. Phosphorylation is a post-translational (ie, after the protein is made) modification of a protein in which a phosphate group is added to a serine, threonine, or tyrosine residue by a kinase (an enzyme that transfers phosphate groups). Proteins are commonly regulated through phosphorylation. Dr. Steffan suspected that phosphorylation in the NT17 region of the huntingtin protein might be important for Huntington's as well and she reasoned that the IKK inflammatory kinase, previously shown to interact with the huntingtin protein, might be involved.

Working with striatal neuron cultures, the Steffan team was able to show that IKK directly phosphorylates Serines 13 and probably Serine 16 (S16). The lack of confirmation of the latter may be because IKK indirectly phosphorylates S16 or because their method of detection may not be sensitive enough to pick up what is happening at S16 but it does appear that Serine 16 is phosphorylated. Analysis of the brains of the R6/2 mice and normal mice detected phosphorylation of the normal huntingtin protein at S13 and S16.

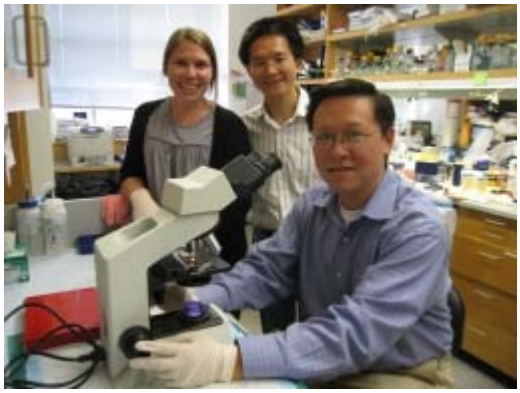
It also appears that IKK phosphorylation causes other postranslational modifications through ubiquitination, SUMOylation, and acetylation. These processes add ubiquitin momomers, small ubiquitin like proteins, and an acetyl group, respectively. This cascade leads to the clearance of the protein in the proteosome and the lysosome.

Lysosomal clearance is dependent on three proteins: the lysosomal-associated membrane protein 2A (LAMP-2A, Heat shock cognate protein 70 (Hsc70), and autophagy-related protein 7 (Atg7). Knocking down either LAMP-2A or Atg7 results in a build up of the huntingtin protein and its fragments, showing that they are necessary for lysosomal clearance. Over-expressing Hsc70 results in increased levels of phosphorylation and acetylation, suggesting that Hsc70 activates the IKK regulated clearance process.

LAMP-2A imports proteins across the lysosomal membrane for chaperone mediated autophagy. For huntingtin's, the chaperone is Hsc70.

The Steffan team also found that phosphorylation enhances nuclear localization. Previous research has shown that huntingtin is normally a cytoplasmic protein; when it accumulates in the nucleus it interferes with gene transcription. The authors note that the phosphorylated protein is less toxic and also suggest that perhaps localization to the nucleus could be a normal part of protein degradation and clearance that is impaired with the expansion of the polyglutamine region.

The significance for Huntington's is that the phosphorylation of the HD protein is less efficient compared to the normal one. In addition, lysosomal degradation is more important for the clearance of the HD protein than for the normal one since over time, the HD protein impairs the proteosome. However, LAMP-2A levels decrease with aging, causing chaperone mediated autophagy to decline.



To determine whether mimicking phosphorylation could be a promising therapeutic strategy, Dr. Yang and colleagues in his lab engineered two types of BAC transgenic (HD) mice and followed them for a year. In one type of HD mouse, serines 13 and 16 were mutated to aspartate. This mimics phosphorylation and essentially makes it efficient and permanent throughout the life of the mouse. In the other type of HD mouse, the serines were mutated to alanine which cannot be phosphorylated.

The mice with the mutation that mimics phosphorylation did not demonstrate the motor and behavioral problems that indicate early neuronal dysfunction nor did they experience neurodegeration. In addition, no large aggregates were detected as would normally be found. These aggregates are a hallmark of the disease so their absence can be a good sign. However, if the disease process is present and the HD protein begins accumulating in the cells, the aggregated form appears to be less toxic than the soluble form.

Dr. Yang did, however, find intermediate sized aggregates which appeared to be intermediate sized Exon 1 aggregates as well as aggregates which appeared to have resulted from an alternate pathway. He speculated that the less developed aggregates could be more easily cleared through the lysosome or the protesome.

"Our study identified a critical molecular switch which lies next to the polyQ mutation in the huntingtin protein," Yang said. "We were surprised to find that subtle modification of only two serine residues in this very large protein can prevent the onset of disease. This finding suggests an exciting new avenue to develop therapeutics for Huntington's disease."

Dr. Yang and colleagues conclude that further molecular analyses of the differences between the HD protein in the mice with the serine mutations that mimic phosphorylation and other forms of the mutated protein can suggest a way to design a high throughput screening assay to identify potential treatments.

The potential of the new research has been recognized at NINDS. "These studies shed light on the structure and biochemistry of the mutant huntingtin protein and on potentially modifiable factors that affect its toxicity," said Margaret Sutherland, Ph.D., a program director at NIH's National Institute of Neurological Disorders and Stroke (NINDS). "They reveal sites within the huntingtin protein and within broader disease pathways that could serve as targets for drug therapy."

Drs. Thompson and Steffan speculate that a treatment which causes or mimics phosphorylation of serines 13 and 16 would need to be offered early, while the protein clearance machinery is still working at top form. When the machinery becomes less efficient with aging and exposure to the HD protein, the treatment could actually become harmful by speeding up the accumulation of the HD protein in the nucleus of the cell. Dr. Yang plans to continue his research with older phosphomimetic HD mice to see how long they are protected from the disease. Dr. Steffan is looking to see if Serines 13 and 16 are phosphorylated in human cells.

References:

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Press releases from NINDS and University of California at Los Angeles.

News commentary from the Alzheimer Research Forum:
http://www.alzforum.org/new/detail.asp?id=2323

- Marsha L. Miller, Ph.D., January 1, 2010