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Adorn That Amino End: Huntingtin Decorated for Destruction

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Just in time for the holidays, research shows that the protein that causes Huntington disease is liberally decked out with phosphates, acetyl groups, SUMO, and ubiquitin—all of which influence its toxicity as well its degradation. Two papers this week describe the importance of phosphorylation of the amino terminus of huntingtin. In the December 24 *Neuron*, scientists from the University of California, Los Angeles, led by first author Xiaofeng Gu and principal investigator X. William Yang, show that mice carrying disease-causing huntingtin are protected from neurodegenerative disease if the protein is pseudo-phosphorylated. In a separate paper, published online by the *Journal of Cell Biology* December 21, researchers at the University of California, Irvine, led by first author Leslie Michels Thompson and principal investigator Joan Steffan, report that this phosphorylation leads to several other modifications, which tag the protein for destruction.

We have found a critical molecular switch for the disease, Yang said. Although the relevance of the modifications in people remains to be seen, the work suggests that targeting these phosphates could eventually lead to therapeutics. Phosphorylation may be protective in terms of activating the clearance of the protein, Steffan said.

Huntingtin is well known to cause disease when it contains an excess of glutamine repeats, but even so, recent data indicate that regions outside its polyglutamine stretch are key for pathology. For example, a polyproline region appears to slow axonal traffic (see [ARF related news story](#)). The first 17 amino acids of the protein have also received plenty of scrutiny lately. This region is involved in cellular localization ([Atwal et al., 2007](#); [Rockabrand et al., 2007](#)) and accelerates huntingtin aggregation ([Thakur et al., 2009](#)). The amino terminus contains two serines and one threonine, potential phosphorylation sites, as well as three lysines that could be amenable to acetylation, ubiquitination, or SUMOylation. In fact, Steffan's previous work showed that the domain can pick up both ubiquitin and SUMO (see [ARF related news story](#) on [Steffan et al., 2004](#)).

Steffan was intrigued by similarities between the huntingtin amino terminus and sequences in ataxin-1, a different disease-related protein that is regulated by phosphorylation. She suspected that phosphates might control huntingtin, as well. She had a suspect kinase in mind: I κ B kinase (IKK) promotes nuclear localization, aggregation, and toxicity of huntingtin ([Khoshnan et al., 2004](#)). Accordingly, the Irvine researchers transfected a huntingtin fragment and IKK into ST14a striatal neuron cultures, then purified the huntingtin. Mass spectrometry showed that the protein was phosphorylated at serines 13 and 16

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Phosphates Key to Pathogenicity

Yang and colleagues, interested in the importance of these phosphorylation sites, took an in-vivo approach. They engineered transgenic mice to express either a phospho-mimetic huntingtin, with the serines replaced by aspartates, or a phospho-resistant mutant frozen in the unphosphorylated state, with alanines instead of serines. The HD model on which the scientists based their mutants ([Gray et al., 2008](#)) exhibits symptoms including movement problems, anxiety, and neurodegeneration. The scientists compared their new mutants with the original Huntington's model and wild-type littermates.

First, the researchers assessed the motor skills of the animals using a rotarod test. Just like the standard HD model, the phospho-resistant huntingtin mice struggled to maintain their balance on the rotating cylinder. The performance of phospho-mimetic huntingtin animals, in contrast, matched that of wild-type mice.

The test for anxiety consisted of observing the amount of time the animals spent exploring a nerve-wracking (to a mouse) lighted area when a safe dark space was also available. The standard Huntington's mice and phospho-resistant animals were nervous, preferring to stay in the dark, while phospho-mimetic and wild-type animals braved the light area more often.

The researchers weighed the forebrains of the different strains to estimate neurodegeneration. By now the results were no surprise: phospho-resistant mice, like the parent HD strain, had lighter forebrains than phospho-mimetic and wild-type animals. Yang was amazed at the magnitude of the differences. Our paper is as clear-cut a result as you can get, he said. All the stars aligned that does not happen too often.

Phosphates First of Many Modifications

For their part, Steffan, Thompson, and colleagues used cell culture systems to further probe events upstream and downstream of huntingtin phosphorylation. In vitro, recombinant IKK phosphorylated serine 13, but not serine 16. Perhaps, the researchers speculated, IKK phosphorylation of serine 13 primes the other serine for phosphorylation by another kinase. Alternatively, their antibody may just not have picked up IKK phosphorylation. The researchers are currently exploring whether the same sites are phosphorylated in human tissue.

The researchers found that phosphorylation was just one of a set of post-translational modifications to the huntingtin amino terminus. Mass spectrometry also detected acetylation at lysine 9, but only in the presence of IKK, suggesting it was dependent on IKK-mediated phosphorylation of the protein.

Next, the researchers made a phospho-mimetic huntingtin with aspartate residues in place of the serines (S13,16D). They used these mutants to probe the addition of ubiquitin and SUMO groups. The phospho-mimetic had less ubiquitination, evidenced by a reduced ladder on Western blots, than the wild-type protein. Similarly, the mutant showed less reaction with a mono-SUMO antibody. Overexpression of IKK with wild-type huntingtin had a similar effect, suggesting that phosphorylation modulates these modifications.

Using GFP-tagged huntingtin, Steffan and colleagues showed that the phospho-mimetic construct preferentially localized to the nucleus, compared to the wild-type. In addition, the protein was found at lower levels than wild-type, suggesting the cell was degrading the phospho-mimetic at a higher rate. When the researchers inhibited the proteasome or lysosome, huntingtin accumulated, suggesting that phosphorylation

and the accompanying other modifications normally cause nuclear localization and degradation.

Phosphates as Pharmaceuticals

The next challenge for the researchers is to work out the role of the phosphorylation sites in vivo and in people, Thompson said. Steffan conjectured that IKK phosphorylates huntingtin, sending it to the nucleus, where it picks up other modifications that label it for proteasomal and lysosomal degradation. In a telephone discussion with ARF, she speculated that in a young, healthy person, this mechanism may keep toxic huntingtin in check. But as people age, the lysosome and proteasome become less effective. Then, huntingtin may accumulate, causing disease.

Yang's work suggests an alternative mechanism, namely that the phosphorylation of huntingtin affects its ability to aggregate. He collaborated with the laboratory of Ron Wetzel at the University of Pittsburgh, Pennsylvania, to assay huntingtin aggregation. Phospho-resistant and normal polyglutamine-expanded huntingtin formed thick, straight amyloid fibrils, whereas the phospho-mimetic polyglutamine-expanded huntingtin made short, thin fibrils. They seem to form more intermediate aggregates, he said. The phosphorylated form of the protein, then, may not aggregate fully, altering the protein's pathogenicity. In the field of neurodegenerative disease, it is not entirely clear whether aggregates are toxic moieties or neutral or relatively protective side products, so the net impact of this altered aggregation is uncertain.

The two mechanisms may be part of a single, larger pathway, Yang suggested. We hypothesize that these intermediate products may be the ones that are more likely to be cleared, he said. Steffan's evidence also indicates that aggregation may be involved. Her group found that when they immuno-precipitated huntingtin from mouse brain, the phosphorylated and acetylated forms were somewhat insoluble. A form of aggregation may actually be involved in the clearance mechanism, she said.

Given the protection afforded to the HD mice, promoting or mimicking huntingtin phosphorylation might be an effective therapeutic strategy, Yang said. Thompson suggested that such treatment might target a kinase or a phosphatase. But she cautioned that such a treatment might only be effective in people whose proteasomes and lysosomes are working at top capacity and are able to degrade the phosphorylated huntingtin.—Amber Dance.

References:

Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, Steffan JS, Thompson LM, Wetzel R, Yang XW. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in mice. *Neuron*. 2009 Dec 24;64:828-840.

Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, Martinez-Vincente M, Arrasate M, O'Rourke JG, Khashwji H, Lukacsovich T, Zhu Y-Z, Lau AL, Massey A, Hoyden MR, Zeitlin SO, Finkbeiner S, Green KN, LaFerla FM, Bates G, Huang L, Patterson PH, Lo DC, Curevo AM, Marsh JL, Steffan JS. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. *J. Cell Biol.* 2009. [Abstract](#)