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NEURODEGENERATIVE DISEASE

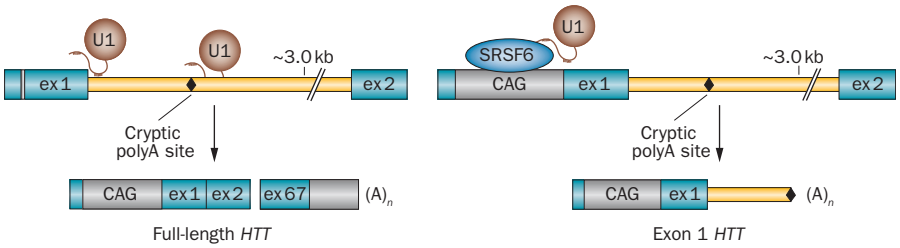
Altered DNA methylation and RNA splicing could be key mechanisms in Huntington disease

March 2013 sees the 20th anniversary of the report that first identified a CAG repeat expansion in the huntingtin (*HTT*) gene as the cause of Huntington disease (HD), but the molecular mechanisms that link mutant HTT protein with neuronal death and dysfunction remain poorly understood. Two papers recently published in the *Proceedings of the National Academy of Sciences* have shed new light on these mechanisms, proposing aetiological roles for altered DNA methylation and RNA splicing.

HD is a hereditary neurodegenerative disorder with motor, cognitive and psychiatric features that generally manifest in middle age. In the absence of a cure for this condition, research efforts are being directed towards the development of strategies to arrest neurodegeneration in the early stages—an undertaking that will rely on improved knowledge of the molecular underpinnings of the disease process.

In the first of the new studies, a team led by Ernest Fraenkel at Massachusetts Institute of Technology, Cambridge, MA, USA examined DNA methylation patterns in mouse striatal cells expressing mutant HTT. "Recent studies show that DNA methylation changes have a role in neuronal activity, learning and memory in healthy brains," Fraenkel points out. "We wondered whether mutant HTT might cause changes in DNA methylation that would then have an effect on cognition."

Using a high-throughput technique known as reduced representation bisulphite sequencing, the researchers discovered altered patterns of DNA methylation in cells expressing polyglutamine-expanded HTT, as compared with those expressing the wild-type protein. The affected genes showed substantial overlap with genes that had previously been found to be dysregulated in the presence of mutant HTT, suggesting that gene expression changes in HD are at least partly attributable to DNA methylation.



Wild-type *HTT* mRNA is spliced correctly (left), whereas mutant *HTT* mRNA undergoes aberrant splicing to produce an mRNA that is translated into a pathogenic exon 1 protein (right). Courtesy of G. P. Bates.

The genes that exhibited both increased methylation and reduced expression included *Sox2*, *Pax6* and *Nes*, all of which encode proteins that are involved in neurogenesis. This finding is consistent with previous reports of impaired hippocampal neurogenesis in animal models of HD.

Fraenkel and colleagues are planning to further explore the clinical applications of their new data. "We are very interested in understanding how these epigenetic changes fit into a large picture of transcriptional regulation, and in using these insights to design new therapeutic strategies," he says.

In the second study, Gillian Bates from King's College London, UK and her co-workers set out to discover the origins of small fragments of HTT that have been identified in samples of brain tissue from individuals with HD. Using a knock-in mouse model of HD, the researchers found evidence of aberrant splicing of the mutant *HTT* mRNA, which resulted in the production of a pathogenic exon 1 protein.

"HTT is a large protein, and considerable evidence has accumulated to indicate that fragments of HTT might be important players in the pathogenic process," explains Bates. "We previously discovered that the smallest fragment present in the brains of HD mouse models is encoded by the first exon of the *HTT* gene—we have found that this is generated through an aberrant splicing event at the level of RNA rather than through the proteolysis of the HTT protein, as had been assumed."

The team went on to demonstrate that the splicing factor SRSF6 binds to the 5' end of the mutant *HTT* transcript. The researchers propose that SRSF6 could influence mRNA splicing by promoting the formation of defective spliceosomes. In addition, this factor might sequester the U1 small nuclear ribonucleoprotein complex, thereby unmasking a polyadenylation signal in intron 1 of *HTT* and enabling an exon 1–intron 1 polyadenylated mRNA to be generated.

"Having identified the origin of this small, highly pathogenic HTT fragment, we are now in a position to prevent its production and determine the extent to which this intervention rescues disease symptoms in mouse models," concludes Bates. "Strategies that target mis-splicing are in active development for many other disorders, and these approaches can now be considered for HD."

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Original articles Ng, C. W. *et al.* Extensive changes in DNA methylation are associated with expression of mutant huntingtin. *Proc. Natl Acad. Sci. USA* doi:10.1073/pnas.1221292110 | Sathasivam, K. *et al.* Aberrant splicing of *HTT* generates the pathogenic exon 1 protein in Huntington disease. *Proc. Natl Acad. Sci. USA* doi:10.1073/pnas.1221891110

Further reading MacDonald, M. E. *et al.* A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971–983 (1993)