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## Discovery Provides New Clues about Causes of Rett Syndrome

Researchers studying the childhood neurological disorder Rett syndrome have discovered a new clue about how the disorder can cause a devastating range of symptoms. They found that MeCP2, the protein that is altered in patients with the syndrome, plays a critical role in snipping and rearranging messenger RNA molecules that carry the genetic code for the construction of other proteins that are important for brain function.

This newly discovered function of MeCP2 offers an additional hint at why the protein is so crucial for development. The finding is helping to round out the picture of MeCP2's complete range of activities, which also include serving as a regulator to repress activity of target genes in the brain.

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— Huda Y. Zoghbi

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The researchers said their discovery offers a promising pathway for understanding the broad and variable symptoms of Rett syndrome, which include language and growth retardation, breathing problems, seizures, motor dysfunction, hand-wringing and social impairment. Furthermore, the discovery may yield insight into a broader range of disorders, including mental retardation and autism, which may also be linked to abnormal MeCP2. Rett syndrome is an X-chromosome-linked disorder that affects about 1 in 10,000 females and is the leading cause of mental retardation in girls.

The researchers, led by Howard Hughes Medical Institute investigator Huda Y. Zoghbi, published their findings in the online Early Edition of the *Proceedings of the National Academy of Sciences*, posted the week of October 17, 2005. Zoghbi and her colleagues are at the Baylor College of Medicine.

In 1999, Zoghbi's research group identified *MeCP2*, or *methyl-CpG binding protein 2*, as the causative gene in Rett syndrome. Zoghbi's team subsequently created a mouse model of Rett syndrome, in which an aberrant *MeCP2* gene produces many of the symptoms seen in humans with the disorder.

According to Zoghbi, MeCP2 was known to represses the transcription of target genes by binding to them and preventing them from transcribing their information into mRNA. However, few MeCP2 target genes had been identified, she said.

“We decided to take a fresh approach to find out more about what the protein implicated in this very complex disorder actually does,” said Zoghbi. “Our hypothesis was that MeCP2 might serve additional functions besides being a repressor.”

Thus, the researchers used a technique called co-immunoprecipitation to identify proteins in the cell that interact with MeCP2. To ensure that any proteins they found were universal MeCP2 partners, they performed their experiments on different types of cells in culture, including neuronal cells, and used different types of “tags” for the MeCP2 protein.

“Whichever way we did this identification, a protein called YB-1 always came up as being associated with MeCP2,” said Zoghbi. Since YB-1, or Y box-binding protein 1, is known to be involved in RNA splicing, the researchers proceeded to explore whether MeCP2 regulates splicing.

RNA splicing occurs when RNA copied from a DNA gene is snipped and rearranged. A process called alternative splicing is critical to the cell's ability to generate an assortment of proteins from the same genes by snipping together different portions of the DNA, and is especially important in development of the brain.

The researchers' experiments revealed that MeCP2 affects RNA splicing through its interaction with YB-1. They also found that mutant MeCP2 interacted less efficiently with YB-1 than did normal MeCP2. In particular, the researchers found that MeCP2 affected splicing of the RNA for a major neuronal receptor, called NR1.

“The finding with NR1 is important, because this receptor seems to be modulated by activity, and we know that MeCP2 level increases in neurons throughout childhood—implying a link to neuronal activity,” said Zoghbi. Thus, she said, the gradual onset of Rett syndrome symptoms in children, who begin developing normally as infants, might be explained by the progressive pathology caused by abnormalities in proteins such as NR1.

Using the mouse model of Rett syndrome, the researchers next searched for splicing alterations in genes that were active in the cerebral cortex. For this experiment, they used a microarray of thousands of such genes supplied by co-author Jason Johnson of Merck. The researchers found that splicing was altered in a significant number of genes from the mutant mice as compared to

normal mice.

“So, we now know that in this mouse model of Rett syndrome, there is quite a bit of RNA splicing alteration in the brain,” said Zoghbi. “The next big question is exactly how these splicing alterations relate to changes in gene expression that might occur in this syndrome. For this disorder, it gets us thinking that regulation of gene transcription and splicing are perhaps coupled and functionally integrated through MeCP2.”

Zoghbi said the next step is to understand how the multitude of abnormalities in RNA splicing caused by mutant *MECP2* contributes to the complex, variable symptoms of Rett syndrome. “What is important is to begin to tie particular molecular changes to a particular clinical feature,” she said. “Once we discover the primary molecular changes that underlie the disorder, we can begin to explore pharmacologic targets to modulate those changes, to alleviate the symptoms of the disease.”