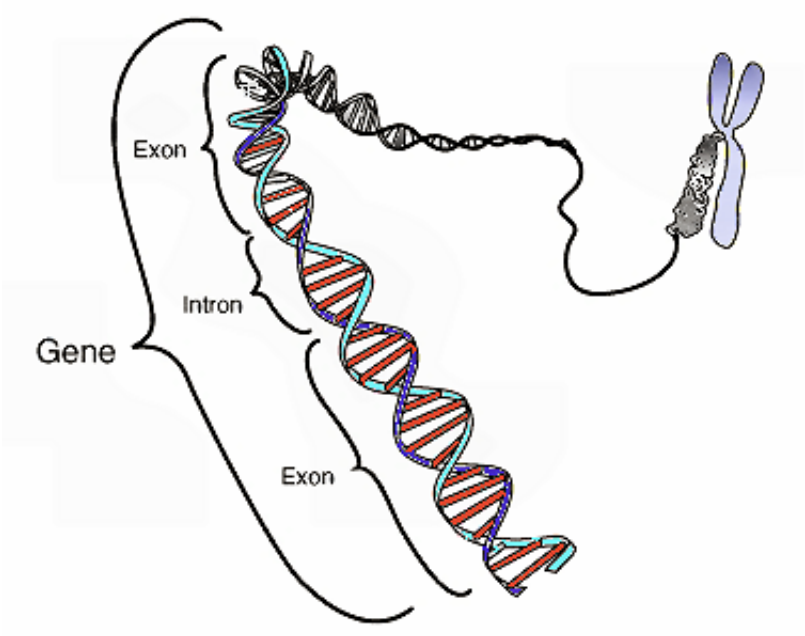
Rett syndrome may result from overexpression of long genes

March 11 2015



This image shows the coding region in a segment of eukaryotic DNA. Credit: National Human Genome Research Institute

Mutations in the methyl CpG binding protein 2 gene (MECP2) are the cause of the devastating childhood neurological disorder Rett Syndrome. Despite intense efforts spanning several decades the precise function of MECP2 has been difficult to pin down. Research primarily funded by the Rett Syndrome Research Trust (RSRT) and the National Institutes of Neurological Disease and Stroke (NINDS), and published today in the journal *Nature* reveals important information that could lead to new

treatment approaches. The study, led by Michael Greenberg, Ph.D., Chairman of the Department of Neurobiology at Harvard University, shows that MECP2 dampens the expression of long genes.

In the early 1990s, Adrian Bird of the University of Edinburgh discovered the MeCP2 protein and proposed that it functions as a repressor of downstream genes. Since then, much effort has been focused on identifying these genes in the hopes that they could potentially become drug development targets. However, the results from numerous labs over the past 15 years have yielded long lists of genes with very little overlap, making it difficult to come to a consensus as to how mutations in MeCP2 lead to neurological dysfunction.

Today's publication sheds new and important light on this puzzle. Researchers Harrison Gabel and Benyam Kinde of the Greenberg lab set out to analyze various gene expression datasets in search of a common theme. This led them to an intriguing finding: the genes disrupted in Rett Syndrome are exceedingly long. The median size gene is about 20,000 nucleotides long, but about 10% of genes are greater than 100,000 nucleotides in length and some extend for more than one million nucleotides. It is the genes that are longer than 100,000 nucleotides that are the most affected in Rett Syndrome.

All of our cells contain the same genes. What differentiates a liver cell from a heart cell from a brain cell are the particular genes that are either silenced or active and the degree of activation, also known as expression.

The researchers in the Greenberg lab found that across all analyzed datasets, and in studies of different mouse brain regions, in the absence of MECP2 the expression of long genes is increased. Furthermore, they found that the longer a gene was, the more it increased. While the increase in expression is modest - only about 3 to 10% - it applies to thousands of genes and therefore might have a significant impact on the

function of the brain.

The scientists gathered additional data in support of the gene length hypothesis. They found that in the biological mirror image of Rett, the MECP2 Duplication Syndrome, long genes are under expressed. They next analyzed long gene expression in mice of different ages. Although pre-symptomatic mice showed detectable overexpression, the effect was more dramatic in symptomatic mice. The researchers also found that the degree of increased long gene expression correlates with disease severity: mice with more severe Rett-like symptoms displayed more overexpression. Finally they looked at gene expression in autopsied brains of individuals with Rett. Just as in the mice models they found that long genes were overexpressed.

Greenberg's lab also found that the disruption of long gene expression appears to be a distinctive signature of Rett Syndrome and related disorders. "When we analyzed gene expression data from other neurological disorders that do not have similarities to Rett Syndrome, we did not see the same effects on very long genes," said Gabel.

The data from the Greenberg lab converge to suggest that Rett Syndrome may result from a relatively subtle yet widespread overexpression of long genes with functions important for the brain while the Duplication Syndrome could be due to under expression of these same genes.

"Interestingly, we found that while all cell types in the body use short and medium length genes, there is more expression of long genes in the brain than elsewhere in the body. This fact could help explain why Rett is mostly a neurological disease," says Kinde.

Last year the labs of Mark Zylka and Ben Philpot at the University of North Carolina at Chapel Hill discovered that a class of drugs called topoisomerase inhibitors reduces the expression of long genes. This

begged the question of whether these drugs could be helpful in Rett Syndrome. Indeed the Greenberg lab found that adding low doses of the drug topotecan to cultured cells lacking MeCP2 normalized levels of long genes. Testing of the drug in mouse models of Rett is now underway.

"MECP2 is one of the most complex problems I have worked on in my career. We persevere because I believe strongly that understanding how this protein works will help us to treat this devastating disorder. It's gratifying to imagine that we, a basic science lab, may have opened the door to a novel way to think about treating Rett and MECP2 disorders. This research was funded primarily by RSRT through the MECP2 Consortium, an unconventional collaboration between our lab and the labs of Adrian Bird and Gail Mandel. We have benefited tremendously from the many conversations with our colleagues in these two laboratories and look forward to aggressively pushing this work ahead," said Michael Greenberg.

Benjamin Philpot at the University of North Carolina, who was not associated with the study, adds, "The work from the Greenberg lab provides further compelling evidence that long genes may be preferentially altered in neurodevelopmental disorders, and in particular in Rett Syndrome and MECP2 Duplication Syndrome. Their tour-de-force project used multiple mouse models, across developmental progression of the phenotypes, as well as human data to convincingly demonstrate a close association between disease severity and the dysfunction of long genes. Thus, this important basic research discovery lays a therapeutic groundwork for treating Rett Syndrome."

More information: Disruption of DNA-methylation-dependent long gene repression in Rett syndrome, *Nature*, DOI: 10.1038/nature14319

Provided by Rett Syndrome Research Trust