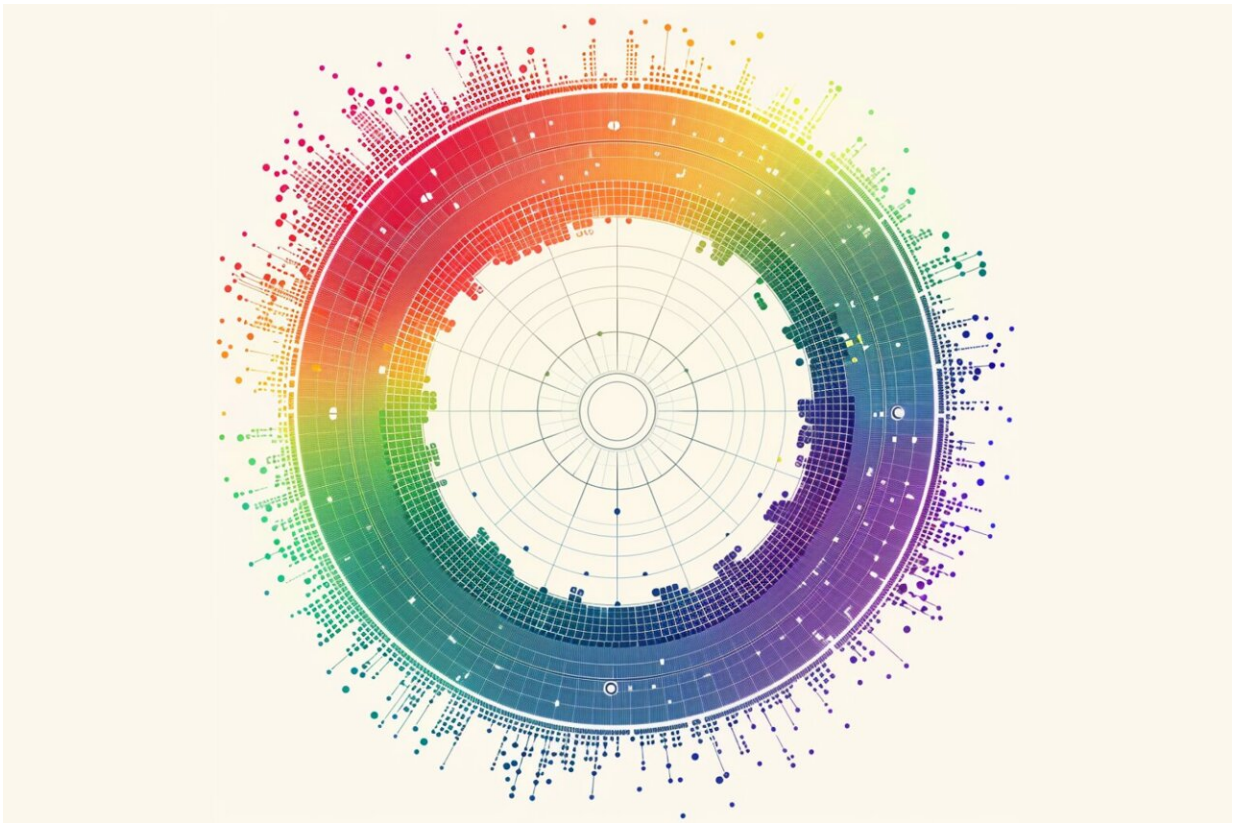# New findings activate a better understanding of Rett syndrome's causes

May 2 2024, by Greta Friar

The integrated epigenome map of MECP2 in normal and Rett syndrome neurons. Credit: Erika Wang/MIT

Rett syndrome is a rare neurodevelopmental disorder for which there is currently no cure or good therapy. It causes severe physical and cognitive

symptoms, including many that overlap with autism spectrum disorder.

Rett syndrome is caused by mutations to the gene MECP2, which is highly expressed in the brain and appears to play important roles in maintaining healthy neurons. The gene is located on the X chromosome and the syndrome mainly affects girls. In order to develop therapies for Rett syndrome, researchers want to understand more about MECP2 and its functions in the brain.

Researchers including Whitehead Institute Founding Member Rudolf Jaenisch have been studying MECP2 for decades, and yet many basic facts about the gene remained unknown. The MECP2 protein encoded by the gene is involved in gene regulation; it binds to DNA and affects the expression level of various other genes—meaning the amount of protein produced from them.

However, researchers did not have a complete list of the genes that MECP2 affects, nor was there a consensus on the effect that MECP2 has on those genes.

Early research into MECP2 suggested that it was a repressor, decreasing the expression of its target genes, but research by Jaenisch and others previously found that MECP2 also acts as an activator, increasing its targets' expression—and that it might primarily be an activator. Also unknown was MECP2's mechanism of action, or what the protein actually does that leads to changes in gene expression.

Limitations in technology had prevented researchers from gaining clarity on these questions. However, Jaenisch, postdoc in his lab Yi Liu, and Jaenisch lab alumnus Anthony Flamier, now an assistant professor at the CHU Sainte-Justine Research Center at the Université de Montréal, have used cutting-edge techniques to answer these outstanding questions about MECP2 and gain new insights into its role in brain health and disease.

Their findings were [published](#) in the journal *Neuron* on May 1, and the researchers have also created an online repository of their MECP2 data, the [MECP2-NeuroAtlas portal](#), as a resource for other researchers.

"I think this paper will drastically change how people think about MECP2 causing Rett syndrome. We have a completely new understanding of the mechanism, and that could provide new ways to approach developing treatments for the disorder," says Jaenisch, who is also a professor of biology at the Massachusetts Institute of Technology.

Fleshing out the picture of MECP2 in the brain

First, the researchers created a comprehensive map of where in the sequence of human neuronal genes MECP2 binds, either within genes or in the regulatory regions of DNA near them. They used an approach called CUT&Tag that can identify protein interactions with DNA with high resolution.

The researchers found more than four thousand genes bound by MECP2. The researchers repeated their mapping in neurons with common Rett-linked MECP2 mutations, to determine where MECP2 is depleted in a disease state.

Knowing which genes MECP2 binds allowed Liu and Flamier to begin drawing connections between MECP2's targets and brain health. They found that many of its targets are involved in the development and functioning of neuronal axons and synapses.
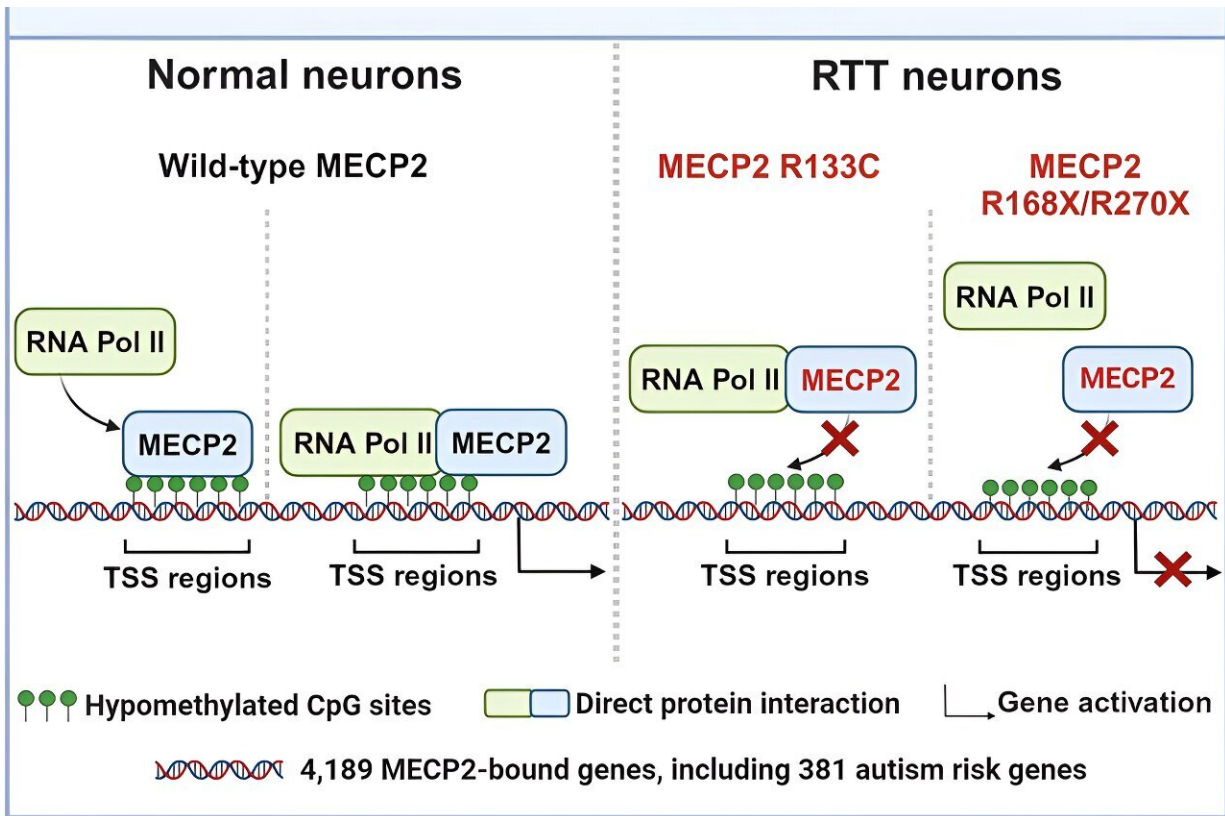
Credit: *Neuron* (2024). DOI: 10.1016/j.neuron.2024.04.007

They also compared their list of MECP2 targets to the Simons Foundation Autism Research Initiative (SFARI) database of autism-linked genes, and found that 381 genes in that database are targeted by MECP2.

These findings may help to narrow in on the mechanisms underlying Rett syndrome's autism-like symptoms, and they provide a good starting point for investigating a possible role of MECP2 in autism.

"We established the first integrated epigenome map of MECP2 in a health and disease state, and this map can guide future research," Liu says. "Knowing which genes MECP2 target, and which genes are directly

dysregulated in disease, provides a strong foundation for understanding Rett syndrome and asking questions about neuronal gene regulation."

The researchers also examined whether MECP2 appears to increase or decrease the expression of its target genes. Consistent with a history of MECP2 being identified by some as an activator and others as a repressor, Liu and Flamier found instances of MECP2 playing both roles.

However, whereas MECP2 is more commonly thought of as a repressor, Liu and Flamier found that it is mostly an activator—confirming earlier findings by Jaenisch and Liu. One new experiment indicated that MECP2 activates at least 80% of its targets, and another indicated that it activates as many as 88% of its targets.

The researchers' map of target genes provided further insight into MECP2's activator role. They found that for the genes that MECP2 activates, it typically binds a region of DNA upstream of the gene called the transcription start site.

This site is where cellular machinery initiates the process of the gene being transcribed or read into RNA, after which the RNA is translated into a functional protein that is the product of gene expression. MECP2's presence at the transcription start site, where gene expression begins, is consistent with its role as a gene activator.

Next, the researchers set out to determine what MECP2's role is in gene activation. They investigated what molecules MECP2 binds to at this site, in addition to the DNA, and found that MECP2 interacts directly with a protein complex called RNA polymerase II (RNA Pol II). RNA Pol II is the key cellular machine that transcribes DNA into RNA. RNA Pol II cannot locate genes on its own, so it requires a plethora of cofactors or collaborator proteins that help it to carry out its job.

The researchers propose that MECP2 serves as one such cofactor, helping RNA Pol II to initiate transcription at the genes where MECP2 binds. Structural analysis of MECP2 identified the parts of the molecule that bind RNA Pol II, and other experiments confirmed that loss of MECP2 decreases the presence of RNA Pol II at relevant transcription start sites as well as expression levels of the target genes.

This suggests that Rett syndrome may be caused by a decrease in transcription of the genes targeted by MECP2, due to MECP2 mutations that prevent it from binding RNA Pol II or binding DNA. Consistent with this idea, most of the common disease-linked MECP2 mutations are truncations: mutations in which part of the protein is missing, which may alter the interaction between MECP2 and RNA Pol II.

The researchers hope that not only will their findings reshape our understanding of MECP2, but that a deeper and broader understanding of how MECP2 impacts brain development and function may lead to new insights that can help people with Rett syndrome and related disorders, including autism.

"This project is a great example of the collaborative nature of the Jaenisch lab," Flamier says. "Rudolf and Yi had a specific problem to solve about Rett syndrome, and I had familiarity with a technology, CUT&Tag, that could solve the problem. Through discussion we realized we could merge our efforts and now we've got a great repository of information on MECP2 and its connections to disease."