The Role of MecP2 Mutations in a Reg-Rett-Able Syndrome

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I. Abstract

Rett Syndrome affects about one in every 10,000 to 15,000 births. It is a genetic neurodevelopmental disorder that mainly occurs in females. Affected males die in infancy because the syndrome exhibits an X-linked dominant pattern of inheritance. Rett Syndrome is caused by mutations in the X-linked Methyl CpG binding protein 2 (MeCP2) coding gene. MeCP2 is necessary for epigenetic regulation of gene expression. This protein represses transcription by acting as a molecular bridge between methylated DNA and a complex of co-repressor proteins including histone deacetylases and Sin3A. Mutations of MeCP2 cause overexpression of several genes during brain development. MeCP2 is a 52-kDa protein with two functional domains the transcriptional repressor domain (TRD) and the methyl-CpG binding domain (MBD). Within the MBD, mutations of Arg106, Arg133, Phe155, and Thr158 (Figure 1) result in a decreased binding affinity of MeCP2 to methylated DNA: 2-fold for mutated Thr158 and 100-fold for the remaining mutated amino acids. The MBD has three beta sheets with Thr158 located on the C-terminal end. Here, hydrophilic residues interact specifically with the methylated DNA. The Hartford Union High School SMART (Students Modeling A Research Topic) Team designed a model of MeCP2 using 3D printing technology to represent the MBD-methylated DNA complex. The model highlights the amino acids involved in the interaction between MeCP2 and methylated DNA. Modeling the structure of MeCP2 allows for a more detailed understanding of the interaction between MeCP2 and DNA. This information will be crucial for designing treatments or interventions to improve the quality of life for Rett Syndrome patients.

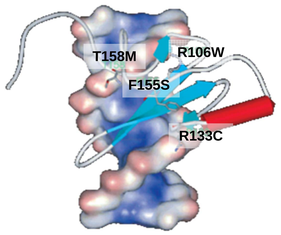


Figure 1$_1$: *This is a model of the locations of the amino acid mutations of MeCP2 and the interactions of the MBD of MeCP2 and methylated DNA.*

II. Rett Syndrome

- Rett Syndrome is an X-linked neurological disorder that causes unusual motor movements, most commonly in the eyes and the extremities. It is a severe disorder as 30% of affected females do not live past the age of 35.
- It is one of the most common causes of cognitive disability in females.
- At just six months of age, symptoms begin to present themselves and often include the loss of coordination, fine motor control, and speech.
- There is no known cure, and patients require lifelong, 24 hour care.

What Causes Rett Syndrome?

- Rett Syndrome is caused by dysregulation of gene expression during brain development.
- The majority of classical Rett Syndrome cases are caused by mutations in *MECP2 gene* (methyl-CpG-binding protein 2), a protein that regulates gene expression via epigenetics.

Figure 2$_3$ (Right) : *Grace has Rett Syndrome. She is unable to walk, talk, use her hands, and eat food on her own.*

III. Epigenetics

Epigenetics is a mechanism that regulates gene transcription by regulating the confirmation of the chromatin without changing the DNA sequence. The confirmation is changed by chemical modification of histones and DNA, the two components of chromatin. MeCP2 binds the methylated DNA and recruits transcription repressors to inactivate transcription (Figure 3). For many genes during brain development, MeCP2 acts as a biochemical switch that either turns on or turns off gene transcription.

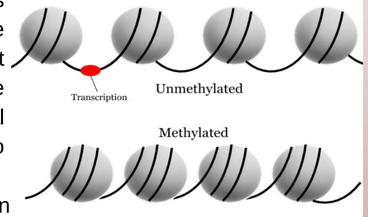
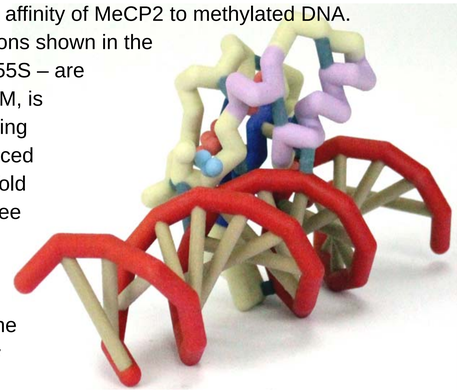


Figure 3 (above): *When DNA is unmethylated (acetylated), transcription factors can turn genes on while methylated genes are tightly packed.*

IV. The MeCP2 Structure Model

Figure 4$_2$ (right) shows a 3D-printed model of file 5BT2.pdb from the Protein Data Bank. The model represents the interaction between the MBD and DNA complex. Four commonly-found MeCP2 amino acid mutations have been shown that result in a decreased binding affinity of MeCP2 to methylated DNA. Three of these amino acid mutations shown in the model – R106W, R133C, and F155S – are colored salmon. The fourth, T158M, is colored sky blue because its binding affinity to methylated DNA is reduced by just 2-fold rather than by 100-fold as in the other mutations. The three beta sheets are dark slate blue, and the two alpha helices are thistle. Hydrogen bonds are tan. When mutated MeCP2 binds to the DNA ligand, genes are wrongfully expressed.



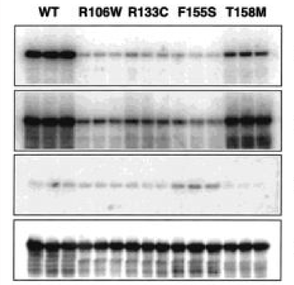
V. MeCP2's Role in Rett Syndrome

MeCP2 functions as a bridging domain necessary for the process of transcriptional repression. The methyl-CpG binding domain (MBD) is one defined domain of MeCP2. A portion of the MBD contains two longer Beta-strands, which interact with the methylated CpG di-nucleotides in the major groove of DNA. The purpose of modeling MeCP2 is to show the interaction between the methylated DNA and MeCP2 binding domain. In addition, it allows for the highlighting of the most common Rett Syndrome mutations, which occur in this MBD. Mutations R106W, R133C, and F155S (salmon in figure 4) reduce MeCP2's binding affinity for methylated DNA by more than 100-fold, and T158M (skyblue) reduces MeCP2's binding affinity for methylated DNA by 2-fold.

Dr. Makky spends a significant amount of time teaching epigenetics. She is interested in this model because she will use it as a teach tool in the classroom. She is particularly interested in the role of X-chromosome inactivation and the upregulation and downregulation of genes in different environments in Rett Syndrome.

VI. Basis for Future Treatments

The 10% polyacrylamide non-denaturing vertical gel shift assay compares the binding affinities of wild type MeCP2 versus mutated MeCP2. The results occurring in this assay are predictive of future treatments for Rett Syndrome by demonstrating that when DNA is hypermethylated (as in GAM12), mutated MeCP2's affinity to bind to the DNA decreases. Hypermethylation would be effective in treatment because the DNA becomes more tightly wrapped and the transcription repression factor can be attached by the wild-type MeCP2. This action reiterates the fact that by hypermethylating DNA, it is possible to prevent mutated versions of MeCP2 from binding to the DNA while keeping the wild-type MeCP2 active. By disabling mutated versions of MeCP2 through hypermethylation, we can ensure that gene expression is controlled; this provides a possible future treatment of Rett Syndrome.



DNA methylated at 12 locations

DNA methylated at 1 location

Acetylated (unmethylated) DNA

Antibodies for MeCP2

Figure 5$_1$

VII. Summary

Even though Rett Syndrome does not affect a large population of society, scientists are concerned about this devastating disease due to its lethal and debilitating effects. Scientists are investigating the four most common mutations that are present in Rett Syndrome patients: R106W, R133C, F155S, and T158M. Their interest stems from the mutations' abnormal binding affinities to methylated DNA. Scientists have yet to understand MeCP2 in its entirety as they develop treatments for Rett Syndrome. Scientists are beginning to wonder if MeCp2 and its binding to methylated DNA could be used to treat Rett Syndrome and other genetic disabilities. Although there are no current effective treatments, the hypermethylation of DNA is a promising pathway for the future.

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