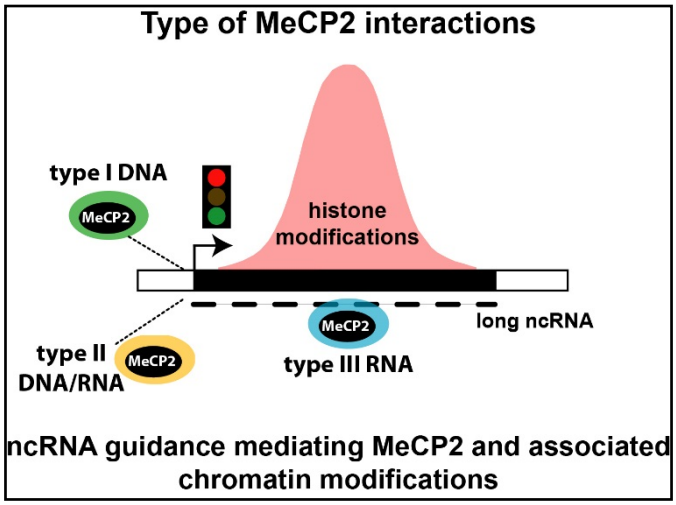
MeCP2 interacting ncRNAs associated with gene regulatory changes

Overview

The discovery that Rett syndrome a neurodevelopmental disorder is caused by mutations that affect the methyl-CpG-binding protein MeCP2 provided a major breakthrough in understanding this severe neurodevelopmental disorder. However, gene regulation by MeCP2 still remains poorly understood. The current consensus for MeCP2 remains as a "classical" repressor complex, and the major emphasis has been on its well-characterized role with respect to methylation-dependent binding and repression. However, recent evidence indicates additional regulatory roles, suggestive of a "non-classical mechanism" of regulation mediating gene activation. Thus, we have questioned whether the classical view of MeCP2 is an accurate reflection of its role in regulating gene expression. Our group is interested to understand the multifaceted nature of MeCP2 interactions, which is classified here as interactions with DNA (type I) as well as DNA/RNA (type II) and RNAs (type III). Thus, we and others, have questioned whether the classical view of MeCP2 is an accurate reflection of its role in mediating chromatin structure and function. The search for, and the discovery of MeCP2 target genes has long been a goal for researchers, with few confirmed targets identified. Indeed, recent genome wide studies show discordant binding not associated with DNA methylation. This now opens the field of MeCP2 research and suggests that the gene targets may not be the usual suspects, i.e. dependent on methylation.

Emerging evidence indicates that non-coding RNA (ncRNA) sequences can drive gene changes and direct transcriptional control. Precisely, how these mechanisms collectively operate in the brain and their relevance to MeCP2 remain poorly understood. Key findings from our laboratory indicate a previously unrecognized mechanism of gene regulation by MeCP2 associated with RNA. Indeed, our own efforts to screen genome wide MeCP2 association using massive parallel sequencing, have identified binding of the MeCP2 protein without exclusivity for methylated CpG. We believe ncRNAs could mediate chromatin-remodeling events, by interacting with MeCP2, thereby conferring gene expression changes in the brain. We have screened for the binding of MeCP2 associated RNAs coupled with our ChIP-Seq (RNA-ChIP-Seq). Some of the most prominent transcripts identified by deep sequencing that were enriched by MeCP2 were ncRNAs. We postulate that MeCP2 interacting ncRNAs can be considered important for gene silencing and activation events in the brain. This research extends the work by our group to show context-dependent reprogramming of the MeCP2 associated SWI/SNF complex.



Hypothesis and Project Aims

We hypothesize that MeCP2 interacting ncRNAs are associated with gene regulatory events and this represents a new paradigm of regulation and histone modification. The specific aims of the project include;

- To determine the role of chromatin modifications in the context of ncRNAs and understand the opposing roles of the MeCP2 regulatory complex in the control of gene expression patterns

- To understand the role of RNA directed MeCP2 mediated gene regulation

The project plan is to identify key gene expression (coding) and non-coding (ncRNA) patterns in the MeCP2 Null mutant using RNA sequencing and its validation by qRT-PCR as well as determine RNA interactions with chromatin as a template for epigenetic modifications. In order to understand histone modifications the project explores gene activating and suppressing events associated with histone acetylation and methylation at H3K4, H3K9 and H3K27 sites using ChIP-Sequencing. We are also investigating ncRNA expression patterns as a result of cortical depolarization. We plan to characterize neurons after depolarization and to determine whether the specific gene regulatory events associated with the ncRNA chromatin interaction involve MeCP2 complex.

Overall Project Outcomes and Significance

This project is designed to address how key regulatory pathways involving MeCP2 are finally converted into an altered gene expression profile that leads to gene deregulation. The findings of this project would further support a new role for MeCP2 interacting with ncRNAs to mediate chromatin remodeling changes and gene regulatory events. Not only is this of direct therapeutic relevance to Rett syndrome, but could also be important for our understanding of new mechanisms of gene regulation conferred by MeCP2 interacting ncRNAs that influence chromatin structure and function.