

## Research Awardees: NIH

2007

Lucas Pozzo Miller

University of Alabama Birmingham

Role of BDNF in dendritic pathologies caused by Rett-associated MeCP2 mutations

\$137,500

### Abstract

The neuropathology of mental retardation is thought to be associated with deficits in synaptic structure and function. Our goal is to make contributions into understanding the formation and maintenance of dendritic spines and of postsynaptic Ca<sup>2+</sup> homeostasis in neurons expressing Rett Syndrome-associated mutations in MECP2. Rett syndrome (RTT) is an X-linked developmental disorder and the leading cause of mental retardation in females. Mutations in the transcriptional repressor MECP2 have been identified in >90% of RTT cases. One of the target genes of MeCP2 is *bdnf*, brain-derived neurotrophic factor. Considering that BDNF has recently emerged as a potent modulator of activity-dependent synaptic development and plasticity in the postnatal brain, including fundamental neuronal properties such as dendritic spine density and form and neuronal Ca<sup>2+</sup> signaling, we hypothesize that a deregulation of BDNF signaling may underlie the dendritic pathologies observed in RTT. The specific hypothesis to be tested is twofold: 1) RTT-associated MECP2 mutations cause dendritic spine loss leading to impaired dendritic Ca<sup>2+</sup> signaling in hippocampal pyramidal neurons through reduced BDNF signaling; 2) impaired dendritic structure in MECP2 mutant neurons can be reverted by BDNF treatment. The consequences of mutant MECP2 expression will be evaluated in neurons maintained in organotypic slice cultures and transfected by particle-mediated gene-transfer. The biolistic gene-transfer approach provides a more flexible way to introduce different mutant forms of MECP2 compared to the generation of transgenic or knockout mice, in addition to allow the co-transfection of other cDNAs or knockdown siRNA constructs of interest. Thus, it represents a novel cellular model of RTT. Transfected neurons will be studied by laser-scanning confocal and time-lapse multiphoton microscopy, as well as by simultaneous Ca<sup>2+</sup> imaging and whole-cell intracellular recordings. This combination of state-of-the-art approaches has never been used to investigate MECP2 function, or applied to animal models of RTT. We expect the proposed studies to provide novel insights into the consequences of mutant MECP2 expression in hippocampal neurons in a cellular model of RTT.

[Back to Top](#)

2006

Terumi Kohwi-Shigematsu

Lawrence Berkeley Lab

Characterization of MeCP2 target genes in Rett Syndrome

\$120,000

## Abstract

Methyl-CpG binding protein 2 (Mecp2/MECP2) is thought to selectively bind methyl-CpG dinucleotides in the mammalian genome and to function as a transcriptional repressor in vivo by interacting with Sin3A and recruiting histone deacetylase (Hdac). Mutations in MECP2 are associated with Rett syndrome, an X-linked neurodevelopmental disorder. We recently found that a maternally expressed imprinted gene, *Dlx5*, is a direct target gene for Mecp2 in mouse brain and in lymphoblastoid cells from individuals with Rett syndrome. *DLX5* showed a loss of imprinting. As a result, *Dlx5* expression is increased 2-fold when Mecp2 is absent or mutated. We also reported that Mecp2 regulates genes by forming a chromatin loop specifically in transcriptionally silent chromatin. *DLX5* regulates the production of (-)-aminobutyric acid (GABA). Using transgenic mice expressing increased levels of *DLX5*, we propose to study whether *Dlx5*/*DLX5* dysregulation per se is sufficient to cause some of the neurological phenotypes of Rett syndrome. We will investigate this question by systematic behavioral analyses of the *DLX5* transgenic mice. We will study expression of *Dlx5* and *Gad*, an enzyme which synthesizes GABA, as well as GABA itself, in various subregions of the brains of wild-type, Mecp2-null, and *DLX5* transgenic mice (Specific Aim 1 and 2). In addition to *Dlx5*, we recently identified another imprinted gene, *Peg3*, to be dysregulated in the hippocampus of the Mecp2-null brain. We will study whether *Peg3* is also a direct target gene for Mecp2, and if it is, we will determine how Mecp2 regulates expression of this gene (Specific Aim 3). Because *Peg3* is known to be linked to maternal behaviors, we will evaluate the maternal behaviors of Mecp2 (+/-) female mice expressing increased levels of *Peg3* in their brain. Furthermore, we will evaluate *Peg3* overexpression in myogenesis by the in vitro differentiation cell culture system (Specific Aim 4). This is because *Peg3* is specifically and abundantly expressed in the brain and skeletal muscle in adult mice. Our study will provide information about the molecular mechanisms underlying Mecp2 activity and how MECP2 mutations might cause Rett syndrome.

[www.hhmi.org](http://www.hhmi.org) (1)

[www.hhmi.org](http://www.hhmi.org) (2)

[Back to Top](#)