

## Research Awardees: 2006

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### Research Awards

Yves Alain Barde

University of Basel

Using neurons generated from embryonic stem cells: MeCP2 targets and BDNF

\$100,000

Research Sponsor: Ford Motor Company

## Lay Summary

Understanding the mechanisms leading to brain dysfunction is a prerequisite for the treatment of disorders such as Rett Syndrome. One of the reasons why it is comparatively more difficult to understand disorders affecting the brain compared with other organs is the remarkable cellular diversity of the brain. Its very function depends on the individual contribution of many different types of cells that are connected to each other. Rett Syndrome is one of the few neurological diseases that can clearly be linked with the abnormal function of a gene (designated MECP2) and for which a very useful animal model exists. Given what has been learned about the function of the defective gene that encodes for a protein with the name of MeCP2 it is very likely that in the absence of normal gene function, the expression of other genes in the brain may be dysregulated. However, it has been very difficult so far to precisely identify those genes which are affected by the lack of normal MeCP2 function. As one of the reasons may reside in the complexity of the cellular system used to perform these analyses (in essence, extracts of brains were used in these experiments) it is hoped that the use of an in vitro cellular system in which virtually all the cells are identical may facilitate the identification of genes that are not expressed at normal levels. As previous work in mouse models has suggested that the expression of one of the major growth factors in the brain, the so-called brain-derived neurotrophic factor (BDNF), may not be regulated normally, we also plan to study the expression of this particular gene in our cell culture system. Indeed, BDNF is required for normal brain development and it is well possible that at least some of the brain alterations seen in Rett Syndrome may be caused by abnormal levels of BDNF.

## Abstract

We recently introduced a cell culture system allowing the generation of a virtually pure and defined population of neural progenitors. Like they do in vivo in the cerebral cortex, these cells go on to differentiate into glutamatergic neurons that form functional synapses. As this system is unique in terms of homogeneity we plan to perform a transcriptional analysis and to compare the expression patterns of transcripts between wild-type and ES cells lacking Mecp2. Given very recent results further strengthening the link between MeCP2 and BDNF, we also plan to examine the biosynthesis of BDNF in neurons generated from wild-type and mutant ES cells. The role of electrical activity and its influence on the biosynthesis will also be examined.

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Adrian Bird

University of Edinburgh

Testing for Phenotypic Reversibility in a Mouse Model of RTT

\$100,000

Research Sponsor: The Hadar Zysman Fund

### Lay Summary

Rett Syndrome patients suffer from microcephaly and have nerve cells with fewer branches due to absence of fully functional MeCP2 protein. An important question for future therapeutic approaches to this disorder is: how reversible are these defects? Can the faulty nerve cells be rescued, or is the damage done during development without normal MeCP2 irreversible? To address this question, we have developed an MeCP2-deficient mouse that mimics many human symptoms of Rett Syndrome, but has the ability to start making MeCP2 again when injected with the drug tamoxifen. We plan to wait until symptoms develop and then switch MeCP2 expression on to see whether or not a healthy brain can be restored.

### Abstract

Our laboratory earlier created a mouse model of Rett Syndrome that showed delayed onset of neurological symptoms resembling the human condition. Mecp2-null males become symptomatic at ~6 weeks and die ~4 weeks later. Mecp2 +/- heterozygotes, which are the genetic model for human RTT, acquire symptoms at 4-12 months and these remain stable. This project will ask whether the phenotypes observed in Mecp2-null and Mecp2 +/- heterozygote animals can be reversed by reactivating the Mecp2 gene. We have created a mouse line that contains an Mecp2Lox-Stop allele that is unable to produce the MeCP2 protein. When combined with a transgene expressing the cre recombinase under tamoxifen control, we have confirmed that the Stop cassette can be deleted at will by drug injection. Reactivation in 3-4 week old mice prevents manifestation of the Mecp2-null phenotype. We now plan to test the reversibility of the phenotype by reactivating Mecp2 after symptoms have arisen. These experiments will establish whether the reduced dendritic arborization and electrophysiological defects that have been observed in symptomatic mice are reversible and irreversible.

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Vania Broccoli

San Raffaele Scientific Institute (Italy)

Production and first analysis of an animal model for the "early seizure" RTT variant: conditional inactivation of the murine Cdkl5 gene

\$100,000

Research Sponsor: Pro Rett Ricerca

### Lay Summary

RTT is a leading cause of mental retardation in females. In 1999, the gene coding for MeCP2 was identified as the molecular cause of RTT. Since then, defects in this gene have been identified in about 80% of classical patients and 20-30% of atypical RTT patient that display differences in disease symptoms and overall severity. The existence of RTT patients carrying normal MeCP2 suggests that other genes can cause RTT. In fact, defects in another gene, CDKL5,

has been very recently identified in some atypical RTT patients. Although, CDKL5 has been shown to bind to MeCP2, its function is still obscure and its role in regulating MeCP2 phosphorylation controversial. For all these reasons, functional studies of this protein are welcome to enlighten molecular relationships between typical versus atypical RTT diseases. We are developing Cdkl5 mutant mice hoping to generate a mouse model of atypical RTT syndrome. These animals will contribute in two ways to research progress in the RTT field. First, they will be instrumental in improving our understanding of Cdkl5 activity in the cell and will shed light on the close relationship between Cdkl5 and MeCP2. Second, these mice will represent a valuable model to evaluate the therapeutic potential of new pharmacological or gene based clinical approaches. We believe these animals will be useful to all the RTT research community and we will grant free access to these animals in the future.

## Abstract

Rett Syndrome is a severe neurological disease with a large impact in the society due to its high incidence in the population. In addition to classical RTT disease associated with MeCP2 mutations, some RTT variants have been described presenting general features of the classic form but with different onset and severity. These variants rarely display MeCP2 mutations, and the genes affected are still mainly unidentified. However, recently, mutations in the Cdkl5 gene have been shown to cause early-seizure RTT variant. These findings open the way to unravel the relationships and possible interactions between MeCP2 and molecules causing RTT variants. Interestingly, Cdkl5 interacts directly with MeCP2 mediating its phosphorylation. However, Cdkl5 is hitherto rather uncharacterized protein. To shed light on Cdkl5 function, we plan to develop mice in which this gene will be conditionally inactivated by gene targeting using the cre-loxP system. Cdkl5 conditional mice, carrying exon 4 flanked by loxP, will be mated with cre-expressing mouse lines in order to inactivate Cdkl5 in a tissue specific manner. This proposal includes also a first characterization of Cdkl5 constitutive mutant mice both at behavioral and morphological levels. Further, on a collaborative basis, the changes in MeCP2 protein localization and morphological levels. Further, on a collaborative basis, the changes in MeCP2 protein localization and post-translation modification will be assessed in Cdkl5 mutant cells. These animals will provide with critical information on the Cdkl5 dependent cellular pathways and disclose the role played by Cdkl5 in regulating MeCP2 activity. Thus, these data will impact also in our knowledge on MeCP2 function and its dependence by specific molecular interactors. Cdkl5 constitutive mutant mice will hopefully develop neurological symptoms described in the patients and, thus, represent an animal model of a RTT variant pathology. If so, Cdkl5 will be a precious model where testing innovative therapeutic approaches to fight the onset and clinical course of the disease.

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Michael Crair

Baylor College of Medicine

Examining the role of MeCP2 in regulating the plasticity and development of synapses and circuits in mouse somatosensory barrel cortex

\$100,000

Research Sponsor: Connors Family Charitable Gift Fund

## Lay Summary

Rett Syndrome is a devastating, progressive developmental disorder that distinguishes itself from other neurological disorders in that brain development appears to be completely normal until about a year after birth, when neurological symptoms emerge and normal postnatal brain development is halted or even reversed. Rett Syndrome is caused by mutations in the gene coding for Methyl CpG Binding Protein, or MeCP2, which is thought to function as a transcriptional

repressor. Transcriptional repressors act to 'silence' specific genes so that their protein product is not produced in a given cell. Rett Syndrome has a fairly broad spectrum of phenotypes, ranging from classic Rett Syndrome (hand wringing, social withdrawal, ataxia, seizures, spasticity, anxiety) to more mild forms that are difficult to distinguish from mental retardation or autism. In this research project, we will use mice with mutations in the gene that causes Rett Syndrome to examine what goes wrong in normal brain development and why. We will specifically concentrate on the postnatal development and plasticity of the cerebral cortex, the part of the brain that is responsible for higher order sensory, motor and cognitive function. This part of the brain is thought to be most severely disturbed in humans with Rett Syndrome. The goal of these experiments is to identify how the development of synaptic 'communication' between neurons is disturbed by mutations in the Rett Syndrome gene, *Mecp2*.

## Abstract

The discovery that loss-of-function mutations in the gene coding for MeCP2 cause Rett Syndrome has revolutionized investigations into the pathogenesis of the disease. With the development of targeted mutations of *Mecp2* in mice that recapitulate the human phenotype, investigations into the underlying brain abnormalities that cause Rett Syndrome are now possible. We propose to use a combination of anatomic, molecular biological and physiological approaches to examine the development of the cerebral cortex in normal and *Mecp2* mutant mice. We propose to do this by examining synaptic and neural circuit function, development and plasticity using in vitro brain slice and in vivo single-unit recording techniques from the somatosensory 'barrel' cortex of normal and *Mecp2*308 mice, a mutant expressing a truncated allele of *Mecp2* that recapitulates many of the phenotypes seen in humans with Rett Syndrome. The goal of these experiments is to identify the synaptic and neural circuit abnormalities that cause the neurologic phenotypes typical of Rett Syndrome, such as the progressive loss of cognitive, motor and social skills.

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Gregory David

New York University School of Medicine

Contribution of altered heterochromatic to the pathogenesis of Rett Syndrome

\$100,000

Research Sponsor: Data Associates

## Lay Summary

RTT is a neurological disorder that primarily affects girls after their first two years of life. Symptoms include mental retardation and the loss of verbal and physical skills. The disease is caused by a defect in a protein known as MeCP2, which binds to DNA at specific regions along the chromosomes. Inactivating MeCP2 in the mouse results in the generation of animals that exhibit Rett-like symptoms. What MeCP2 does on the DNA is still unclear, but it has been proposed that MeCP2, through its interaction with other proteins known as the Sin3 proteins, can repress the expression of some factors that remain to be identified. Therefore, RTT could be the result of aberrant expression of proteins that should not be present in neurons when MeCP2 is not mutated. Identifying those factors and being able to modulate their expression using drugs is a priority to cure RTT. In the adult organism, cells must stop proliferating and differentiate to perform their ultimate function, and this event results from the actively repressed expression of pro-proliferating proteins. This proliferation stop is highly regulated, and its disruption is detrimental for the function that the cell was genetically programmed to perform. We propose here that RTT results from the inability of neurons to maintain a stopped proliferation status, due to the mutation of the MeCP2 protein. To test this hypothesis, we generated mice that do not express the Sin3B protein in neurons, a protein that we have shown necessary for the repression of pro-

proliferating genes. By comparing the behavior, the anatomy and the molecular events that take place in those animals to the ones in MeCP2 deficient animals, we should be able to understand the defects that are responsible for the neurological syndromes in Rett, and design targeted therapies.

## Abstract

MeCP2 is mutated in over eighty per cent of the patients affected with Rett syndrome and its inactivation in mature neurons is sufficient to recapitulate most of the phenotypes associated to the disease in mouse models. The functions of MeCP2 in the normal physiology of neurons remain unclear, but it was recently demonstrated that MeCP2 recruits components of the Sin3 co-repressor complex to repress transcription. However, what are its target genes and how their deregulation contributes to Rett syndrome remain elusive. During terminal differentiation, pro-proliferating genes must be stably silenced to prevent re-entry into the cell cycle. Our preliminary results suggest that Sin3B, a component of the Sin3-HDAC complex, is essential for irreversible cell cycle exit, and limited analysis suggests that Sin3B inactivation in mature neurons may induce Rett syndrome-like phenotypes. We hypothesize that Rett syndrome results at least in part from the inability of the affected neurons to efficiently repress pro-proliferating genes. Our specific aims are to recapitulate Rett syndrome in a large cohort of mice deleted for Sin3B in mature neurons, and to characterize in vivo the neurological and anatomical defects of these animals as they relate to Rett syndrome. The transcriptional repression of pro-proliferating genes in wild-type and Sin3B mutant neurons will then be assessed and compared to MeCP2 mutant brains. Finally, we will take advantage of an in vitro differentiation assay to genetically dissect the transcriptional repression pathways driven by MeCP2 and/or Sin3B at the chromatin level, using ChIP for heterochromatin marks. Altogether, these experiments should enable the delineation of the mechanisms that lead to stable cell cycle exit in differentiated neurons and how its disruption relates to Rett syndrome.

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John Greer/Gregory Funk

University of Alberta

Investigation of Respiratory Dysfunction in Mouse Models of RTT

\$100,000

Research Sponsor: Michael and Jane Joyce

## Lay Summary

RTT is a severe neurological disorder that is frequently associated with a specific genetic mutation. RTT patients suffer from a number of behavioral disorders including severe breathing irregularities. Scientists have developed mouse models with defects in the same gene as in children with RTT. These mice have many of the same symptoms. In this research proposal, Drs. Greer and Funk will collaborate and use 2 distinct RTT mouse models to try to understand what specific aspects of brain function are abnormal to cause the breathing problems. They will also test a number of pharmacological agents to see if they can normalize the breathing pattern.

## Abstract

**Objectives:** To systematically investigate the neural control of respiration in genetic mouse models of Rett Syndrome.

**Hypothesis and Specific Aims:** We will use two distinct mouse models of Rett Syndrome to achieve the following three major specific aims.

**Aim 1:** Characterization of respiratory pattern in mouse models. It will be essential to establish a firm understanding of the respiratory phenotype in the mouse models. We will start by using dual-chamber whole-body plethysmographic recordings to monitor respiratory pattern from the neonatal period onwards, including hypoxic (isocapnic and hypocapnic) and hypercapnic ventilatory responses.

**Aim 2:** Anatomical analyses of medullary structure in mouse models. Histological and immunohistochemical labeling will be performed to examine major brainstem nuclei and axonal projections, including the region of the putative inspiratory rhythm generator (preBotzinger complex, the PBC) before and after the appearance of obvious respiratory dysfunction.

**Aim 3:** Analysis of respiratory rhythm generating networks and their modulation in mouse models. The respiratory abnormalities associated with RTT are state-dependent. The fact that there are extended periods of normal breathing (i.e. during sleep but also during wakefulness) is the basis for the longstanding hypothesis that the respiratory rhythm generator per se can function normally, but during wakefulness receives inappropriate modulation from CNS regions that destabilize the basic rhythm. More recently, it was shown that brainstem aminergic modulatory systems may also be compromised. The fact that these aminergic systems are normally quiescent in sleep therefore raises the possibility that the abnormal rhythm during wakefulness in RTT reflects not only a descending destabilizing influence, but the absence of a stabilizing modulatory input from the brainstem. Rhythmically-active medullary slice preparations from mice between 2 and 3 weeks of age will be used to assess cellular mechanisms and modulator effects at stages when the respiratory phenotype is just emerging. The perfused heart in situ preparation, however, will be the main focus of this aim. With this preparation we will assess central respiratory activity in reduced preparations: i) over a wider development window that covers presymptomatic periods through to end-stage disease; ii) before and after removal of midbrain and pontine structures (possible sources of destabilizing input); and, iii) before and after inhibition or facilitation of brainstem modulatory networks that influence respiratory rhythm.

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Mark Groudine

Fred Hutchinson Cancer Research Center

In vivo characterization of MeCP2B protein interactions in human neuronal cells by quantitative FT-ICR mass spec

\$55,000

Research Sponsor: Robert and Adele Schiff Foundation

[Lay Summary](#)

Up to 80% of RTT are caused by mutation in MECP2. The disastrous neurodevelopmental mishaps in RTT arise from the disruption of the enigmatic and perhaps subtle mechanism by which the normal MeCP2 protein works. A growing body of scientific evidence suggests that MeCP2 is a multifunctional protein that has the potential to act differently

depending on the underlying molecular and cellular context. The studies outlined in this proposal will provide novel insight into the molecular basis for RTT. Furthermore, delineating the molecular mechanism(s) by which MeCP2 causes neuronal dysfunction, may provide insights into other neurological disorders in which aspects of RTT phenotype are manifest, such as autism, Alzheimers, Parkinson's and schizophrenia.

## Abstract

The MeCP2 gene encodes a multifunctional methyl-CpG binding protein that represses transcription of methylated genes in vitro, however, the biological function(s) of MeCP2 in vivo remains unclear. Although MeCP2 is ubiquitously transcribed, recent genetic studies have established that MeCP2 performs a crucial role in the developing postnatal nervous system. Consistent with these findings, it has been proposed that mutations in this gene contribute to the neurodevelopmental disorder called Rett Syndrome (RS). Thus understanding the biological role of MeCP2 in neurogenesis and neuronal maturation may provide insights into how mutations in this gene are involved in the pathogenesis of RS.

Given the broad distribution of Rett (RT) mutations within the MeCP2 coding sequence, we hypothesize that the Transcriptional Repressor Domain (TRD) of MeCP2 mediates multiple, context-dependent intermolecular interactions. Defining the molecular and functional consequences of these spatial and temporal interactions is a prerequisite for understanding the molecular basis of RS. Moreover, manipulation of such mechanisms in the correct context may be of considerable therapeutic value. To test this hypothesis, we propose the following:

**Aim/Objective:** To determine the molecular significance of RT mutations in the MeCP2 coding sequence using a novel in vivo biotinylation tagging and quantitative proteomics cell based assay. Proteins most often interact with each other (as well as with DNA, RNA or metabolites), to form transient or stable complexes which carry out diverse biological activities. Defining the composition of normal and mutant MeCP2 protein complexes, as well as understanding how these complexes are assembled and regulated during neuronal development will yield invaluable insights into the biological function of MeCP2.

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Rudolf Jaenisch

Whitehead Institute

Identification of critical time points for treatment in RTT disease progression and genome-wide analysis of distribution of MeCP2 in gene promoters regions

\$100,000

Research Sponsor: Rett Syndrome Association of Massachusetts

## Lay Summary

Girls affected by RTT get diagnosed only after the first symptoms appear. Can we treat these patients or have they accumulated damage that is not reversible? Previous results from our lab and others suggest that it might be possible to impact the disease progression after birth. To follow up on these observations we are developing a system in mice that



will allow us to re-introduce MeCP2 into diseased animals at any point after birth. This will help us to better understand the disease progression and will tell us if a cure is possible after the first diagnosis or if we need to think about perinatal intervention.

MeCP2 is a transcription factor, which means that it controls the expression of other genes by binding to their DNA. Exactly which genes does it bind to and regulate? To find out we will use antibodies that can bind to the MeCP2 protein and this will allow us to isolate the MeCP2 protein and the DNA that it is bound to. All the DNA fragments that we collect using this technique will be matched to a microarray chip containing the entire mouse genome sequence. The genes on the array are annotated so that when one of our fragments matches a spot in the array we will know that MeCP2 was bound to that gene. Identifying these genes will give us a better understanding of the symptoms and could help us think about new potential drug targets or treatment options.

## Abstract

Identification of critical time points for treatment in Rett syndrome disease progression and genome-wide analysis of distribution of MeCP2 in gene promoters regions. One of the most important issues of Rett research is whether disease progression can be halted or reversed. In order to test this possibility we propose two types of experiments. 1. Using an inducible transgene we will activate MeCP2 in mutant animals at various times before and after the development of symptoms. In the converse experiment we will inactivate MeCP2 at various post-natal times. With these manipulations we aim at defining the time points that are critical for rescuing the phenotype and/or for causing it. 2. In order to understand the pathway to the disease, it is essential to identify the genes whose expression is directly regulated by MeCP2 versus those that are misregulated by secondary effects of the disease progression. To address this issue we will perform ChIP-Chip experiments in which chromatin immunoprecipitation (ChIP) is combined with whole-genome DNA microarrays (Chip) to create high resolution maps of the in vivo interactions between MeCP2 and DNA.

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James Kadonaga

UCSD

Dynamics and Function of MeCP2 in Chromatin and Transcription

\$100,000

Research Sponsor: Reading Rock, Inc.

## Lay Summary

The growth, development, and maintenance of our cells are dependent upon the proper regulation of our genes, which are distinct regions in our DNA that carry out specific functions in the cell. There are special regulatory factors in our cells that control whether each of our tens of thousands of genes is on or off at the correct time and place. One of these regulatory factors is a protein called MeCP2. MeCP2 is a particularly important factor, because mutations (unexpected changes) in the MeCP2 protein are responsible for RTT. The objectives of the proposed experiments are to learn more about how MeCP2 functions in the cell, and importantly, to identify what activities of MeCP2 are altered by the mutations that cause RTT. To address these questions, we will reconstitute (in the test tube) the natural forms of genes, termed chromatin, as they are found in the cell. We will then examine the effects of MeCP2 upon chromatin structure. We will also explore a novel mechanism by which MeCP2 might control gene activity. These studies should provide a

new knowledge that would be applicable toward the development of a treatment or a cure for RTT.

## Abstract

The aim of the proposed project is to understand the fundamental biochemical activities of MeCP2. Because mutations in the human MECP2 gene cause Rett syndrome, there is a critical need to illuminate the function of the MeCP2 protein. We would use our expertise in chromatin assembly and analysis to investigate the effects of wild-type and mutant human MeCP2 proteins upon chromatin structure and dynamics. We would also explore a novel deacetylase-independent mechanism of transcriptional repression by MeCP2. This new knowledge should contribute significantly toward the development of a treatment or a cure for Rett Syndrome.

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Charlotte Kilstrup-Nielsen

University of Insubria (Italy)

Functional characterization of CDKL5, a novel gene involved in the onset of RTT

\$100,000

## Lay Summary

RTT is a neurological disorder and is one of the leading causes of mental retardation in girls. The classic form of RTT is in most cases caused by mutations in a gene called MECP2, which has important functions in the brain since it is involved in regulating the expression of other neuron-specific genes. This has led to the search for other genes involved in causing the disease and recently a novel gene, CDKL5, was found mutated in some patients with the so-called Hanefeld variant, which is characterized by the appearance of epileptic crisis very early in the disease. When mutations in CDKL5 were discovered in RTT patients, this gene was basically unknown. Researchers could only guess some of its functions and one of the assumptions was that it might collaborate with MeCP2, the other gene causing RTT, since mutations in the two genes cause the same disease. We wanted to understand if CDKL5 and MeCP2 really do collaborate and recently, we published results showing that the two proteins actually do interact physically. Based on our experiments, we propose that CDKL5 regulates the activity of MeCP2. However, even if these results suggest that mutations in CDKL5 cause RTT because MeCP2 is not properly regulated, we still need to understand the precise role of this gene. For example, we want to know more precisely how MeCP2 is regulated by CDKL5 and furthermore we think that CDKL5 regulates not only MeCP2 but also other factors that are important for the correct function of the brain. With this proposal we therefore intend to characterize in more details CDKL5; in particular we will study how CDKL5 is regulated, how it regulates MeCP2 and, eventually, reveal some of its role in the nervous system. We believe that this will be an important step towards understanding its role in human health and, in particular in the pathogenesis of RTT.

## Abstract

Rett Syndrome, an X-linked neurological disorder, is one of the leading causes of mental retardation in females. Mutations in the MECP2 gene, located at Xq28, has been found in the majority of patients affected by the classic form of Rett but in less than half of the patients with one of the variant forms of this disorder, suggesting that other genetic loci are involved. Accordingly, mutations in another X-linked gene, cyclin-dependent-like 5 (CDKL5), have recently been identified in some patients with the Hanefeld variant, also called the early onset seizures variant. When CDKL5 was identified as the cause of Rett syndrome, it was an almost completely uncharacterized gene. The similar phenotypes

caused by mutations in MECP2 and CDKL5 suggest that the two genes might work in common molecular pathways. Our recently published results showed that, besides sharing an overlapping expression patterns in the brain, MeCP2 and CDKL5 interact and, importantly, that the kinase activity of CDKL5 mediates the phosphorylation of MeCP2. However, even if these results suggest that CDKL5 plays an important role in the proper functioning of the nervous system by regulating MeCP2 activities, we still need to learn more about the biological role of the novel kinase. For example, since mutations in CDKL5 can be correlated with a particular Rett variant we believe that this gene also has important MeCP2-independent functions, a hypothesis that seems to be confirmed by the fact the CDKL5, but not the Methyl-binding protein, is also involved in West syndrome. The proposed research is therefore aimed at shedding light on the functional role of CDKL5 both as a regulator of MeCP2 activities as well as neuronal differentiation. We believe that a better comprehension of the molecular functions of this new kinase is an essential step towards understanding its role in human health and, in particular, in the pathogenesis of Rett syndrome.

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Jan-Marino Ramirez

University of Chicago

The effects of aminergic reuptake inhibitors on erratic breathing in MECP2 mutant mice

\$100,000

## Lay Summary

One of the most debilitating symptoms in patients with RTT is erratic breathing. Symptoms include hyperventilation, apnea, apneusis and breath holding. To date there is no treatment for these potentially very dangerous symptoms. More than 85% of identified girls with RTT have mutations in the gene encoding MECP2 on the X chromosome. Mice with a MECP2 mutation exhibit similar breathing irregularities as RTT patients. In these mice we previously demonstrated deficiencies in two biogenic amines: serotonin and norepinephrine. These substances are continuously released into an area in the brainstem that controls breathing. When this breathing center (pre-Botzinger complex) is removed in thin slices, the isolated breathing center continues to generate a respiratory rhythm "in vitro". This powerful in vitro approach can be used to test the disturbed cellular mechanisms that underlie erratic breathing in MECP2 mutant mice. Using this preparation we were able to demonstrate that (a) serotonin and norepinephrine is required for regular breathing that (b) a deficiency of these substances leads to erratic breathing, which is explained by the malfunctioning of neurons that are called "pacemaker neurons", and that (c) exogenous application of norepinephrine can produce a regular breathing rhythm in slices of MECP2 mice. However, it is unclear whether these in vitro data are also relevant for the intact animal. In this research project we will therefore test our hypothesis in intact alert animals (aim1). We will specifically increase the endogenous concentrations of serotonin and norepinephrine using so called reuptake inhibitors. Many of these substances are clinically used as anti-depressants or drugs to treat ADHD. We hypothesize that these uptake blockers will regularize breathing in these mice and potentially also enhance their life span. We will also be able to assess if the effects of these reuptake inhibitors wear off. In a second aim we will investigate the cellular bases of this effect, by applying these uptake blockers in slices containing the breathing center. We will also test the effects of these substances in a so called "working heart brainstem preparation", a preparation that contains large portions of the neuronal network that controls breathing. We hope that the combined in vivo and in vitro approach will identify potential therapeutic substances that in the future can be used to pharmacologically control erratic breathing in RTT patients.

## Abstract

Our preliminary data indicate that medullary norepinephrine (NE) and serotonin (5-HT) is deficient in a mouse model

(strain B6.129P2(C)-Mecp2 tml-1Bird) for Rett Syndrome. This deficiency can explain erratic breathing, which is one of the most debilitating symptoms characteristic for mutant mice, but also for patients with Rett Syndrome. Our in vitro data indicate that a specific population of medullary pacemaker neurons, the so-called cadmium-insensitive (CI) pacemaker neurons, is critical for the generation of stable breathing. These pacemaker neurons depend on the persistent sodium current and require endogenous activation of 5-HT<sub>2A</sub> and alpha-2 adrenergic receptors. Blockade of these receptors abolishes bursting in CI pacemakers and leads to irregular respiratory activity resembling erratic respiration in Mecp2 mutant mice. Moreover exogenously applied NE can regularize respiratory activity in Mecp2 mutant mice. The proposed research has two specific aims that will test two hypotheses: Specific aim 1 tests the hypothesis that serotonin-specific, norepinephrine-specific and mixed serotonin-norepinephrine uptake blockers will regularize erratic breathing in alert in vivo Mecp2 mice. Experiments are proposed to test the effect of a variety of commercially available anti-depressant uptake inhibitors in mutant and wt mice using plethysmographic recordings. These experiments will directly assess whether these effects are beneficial, and whether these effects wear off. Specific aim 2 examines the effects of these reuptake blockers in /in vitro /slice preparations and the working-heart brainstem preparation to unravel the underlying cellular mechanisms.

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Gordon Shepherd

Northwestern University

Role of MECP2 in neocortical synaptic circuit organization

\$100,000

[Lay Summary](#)

The cortex of the brain may be a primary locus of the synaptic circuit pathology caused by deficiency of the protein MeCP2, mutations in which cause RTT. Cortical synaptic circuits are at once both among the most abundant circuits in the brain and among the most difficult to characterize, due to the complexity of neuronal interconnections. New imaging and molecular tools now enable detailed study of these pathways in MeCP2-deficient circuits of the cortex including not only well-studied sensory areas but also frontal areas, where cortical circuits underlie a wide range of cognitive behaviors including motor control, decision making, and memory. We will investigate the effects of silencing the expression of MeCP2 in subsets of cortical neurons. The results are expected to have direct implications for understanding the critical "wiring" defects in the cortex of the brain in RTT. Furthermore, we will perform experiments in which we selectively replace MeCP2 proteins in a subset of cortical neurons in MeCP2-deficient mice. This will enable us to evaluate to which cortical synaptic circuit abnormalities can be rescued by re-introduction of normal MeCP2.

[Abstract](#)

We propose an experimental program aimed at elucidating the roles of MeCP2 in neocortical synaptic circuit organization using model experimental systems for Rett Syndrome. Our specific goals are (1) to identify the functional abnormalities in neocortical synaptic circuits caused by MeCP2 deficiency, at the level of individual pyramidal neurons; and (2) to attempt to 'rescue' the synaptic circuit disorders identified in #1 by restoring MeCP2 expression in individual MeCP2-deficient neurons. Among the tools we will apply towards these goals are in utero electroporation of plasmids encoding small interference RNAs to silence MeCP2 expression in a subset of neocortical neurons, MeCP2-mutant mice, and laser scanning photostimulation, a high-sensitivity, high-bandwidth electrophysiologically based imaging tool for mapping synaptic connectivity. Using these tools we will determine the effects of MeCP2 deficiency on the local circuit organization of pyramidal neurons in the neocortex.

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Hongjun Song

Johns Hopkins University

Roles of MeCP2 in regulating synaptic integration and maintenance of newborn neurons in the adult brain

\$100,000

Research Sponsor: Michael and Kelly Fedak

### Lay Summary

One of the central dogma in neuroscience, since the time of Ramon y Cajal, declared a lack of new neuron generation in the postnatal mammalian brain. This long-standing dogma was finally put to rest in the last decade by repeated demonstration of active adult neurogenesis, the process of generating neurons from neural progenitors, in specific brain regions of all mammals examined, including humans. One of the regions with active neurogenesis throughout life is the dentate gyrus of the hippocampus, a brain structure important for learning and memory. While the exact function of adult neurogenesis remains elusive, rapidly accumulating evidence supports important roles of adult neurogenesis in certain type of learning, memory and mood regulation. Recent studies, including ours, have demonstrated that adult hippocampal neurogenesis recapitulates neuronal developmental process in a mature central nervous system (CNS) environment, from proliferation and neuronal fate specification of neural progenitors to migration, targeting and synaptic integration of newborn neurons. The existence of active adult neurogenesis not only reveals a striking regenerating capacity of the mature mammalian CNS, but also provides a model system to understand basic mechanisms regulating distinct steps of the neurogenesis process, from progenitor biology to synapse formation and maintenance in a mature CNS environment. Understanding of basic mechanisms underlying adult neurogenesis will not only provide us basic knowledge of neuronal development in general, but may also reveal the etiology and pathology of some neurological diseases, such as RTT. More importantly, these studies may lead to novel treatment of such diseases. Studies of human patient samples and animal models of RTT suggest that MeCP2 mutations may disrupt the formation, maturation, and/or pruning of synapses. We propose in the current project to examine the role of MeCP2 in regulating synapse formation, maturation and maintenance of newborn neurons in the dentate gyrus of adult mice in vivo using our recently developed model system.

### Abstract

Rett syndrome is an X-linked dominant disorder caused by loss-of-function mutations in the gene encoding methyl CpG binding protein 2 (MeCP2). Studies of human patient samples and animal models of Rett syndrome suggest that MeCP2 mutations may disrupt the formation, maturation, and/or pruning of synapses. The underlying cellular and molecular mechanisms of MeCP2 in these processes are largely unknown. In the dentate gyrus of the hippocampus, new granule neurons are continuously generated from adult neural stem cells throughout life in all mammals examined, including humans. We have recently characterized the neuronal integration process of newborn granule cells in the adult mouse hippocampus, including GABAergic synaptogenesis, glutamatergic synaptogenesis and plasticity. Interestingly, while adult neurogenesis recapitulates neuronal developmental process in a mature central nervous system environment, the neuronal integration process for adult born neurons is significantly prolonged compared to those during fetal development. This offers a unique opportunity to examine in detail the sequential events involved in the formation, maturation and maintenance of both GABAergic and glutamatergic synapses in vivo. We have also developed retrovirus-based strategies for "loss-of-function" and "gain-of-function" in vivo analysis of genes of interest in individual newborn neuron by multiphoton confocal imaging and electrophysiology. In the current project, we propose to examine the cell autonomous and postsynaptic roles of MeCP2 in regulating GABAergic and glutamatergic synapse

formation, maturation and maintenance of newborn neurons in vivo.

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Juan Young

Centro de Estudios Científicos-CECS (Chile)

MeCP2's function in post-translational regulation of gene expression

\$100,000

Research Sponsor: Richards Barry Joyce & Partners, LLC

### Lay Summary

RTT is caused by mutations in the gene encoding for the protein MeCP2. It has been shown that MeCP2 is a "master protein" that regulates the expression of other genes. Genes generate (express) proteins in a multi-step process. The first, called transcription, involves the synthesis of RNA. The second step entails maturation of this RNA by removing parts (introns) and rejoining the remaining fragments (exons), a process called splicing. This RNA (composed of exons) is finally translated into proteins. Notably, the same RNA could be sliced in alternative ways, depending on which exons are included or excluded. Thus, the same gene could finally generate more than one protein through this route of alternative splicing.

There is convincing evidence that indicates that MeCP2 regulates gene expression at the level of transcription. In addition, we found that MeCP2 also functions as a regulator of splicing. We now propose to characterize this newly identified function of MeCP2 and to find direct targets of MeCP2-mediated splicing regulation. This study will help in linking particular molecular changes to particular clinical features.

### Abstract

Rett syndrome (RTT) is a disabling neurodevelopmental disease that involves loss of cognitive, motor and social skills, whose pathogenesis remains far from being completely understood. It is caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2), a methylation-dependent transcriptional repressor. I would test the hypothesis that methyl-CpG-binding protein 2 (MeCP2) is another example of multifunctional-multi-level regulator of gene expression. Support for this hypothesis comes from our finding that MeCP2 interacts with proteins involved in RNA processing. Further, our data suggest that MeCP2 performs a splicing related function, in addition to its role as a transcriptional repressor. The proposed study is designed to determine MeCP2's functions and their participation in RTT pathogenesis. We propose to test the hypothesis that MeCP2 is involved in the regulation of RNA processing, via its interaction with RNA and RNA processing factors. In particular, we propose to evaluate the functional consequences of the interaction of MeCP2 with RNA and YB-1, to determine the mechanistic basis of its splicing-related activity and to identify targets of MeCP2-directed splicing regulation.

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Xinyu Zhao

University of New Mexico

Effect of MeCP2 mutation in neuronal maturation and function in heterozygote females

\$100,000

### Lay Summary

RTT is first manifested in 6-18 month old children, correlating with a major period of postnatal neuronal maturation. Using mice lacking functional MeCP2, we have determined that MeCP2 is important for neuronal maturation. However, most RTT patients are heterozygote females and are mosaic in MeCP2 deficiency due to random inactivation of the X chromosome. This means that about 50-80% of the neurons in their brains have functional MeCP2. Since neuron-neuron interactions are important for neuronal development and function, it is possible that MeCP2 mutant neurons act as a "bad neighbor" and can negatively affect the maturation and function of normal MeCP2 expressing neurons. This negative effect could be responsible for the progression of neurological deficits in RTT patients during both the initial onset of symptoms and late stage deterioration. The goal of this proposal is to test the hypothesis that in heterozygote females mutation of MeCP2 leads to abnormal inter-cellular signaling, which results in defective maturation and function in neurons expressing wild type (normal) MeCP2. To test this hypothesis, we propose to first characterize the maturation and function of neurons expressing functional MeCP2 in a novel MeCP2 mosaic mouse model that contains wild type and mutant neurons readily distinguishable by GFP fluorescence; and second, to characterize the effects of MeCP2 mutant neurons on wild type neurons in co-culture assay. If we determine how MeCP2 mutation affects the development and function of wild type MeCP2 expressing neurons in heterozygote females, we can then develop novel therapeutic approaches by blocking the negative effect of mutant neurons on normal MeCP2 expressing neurons, or supplying these neurons with the factors that are limiting in heterozygote brains. Ultimately, this research may identify the means to stop or reverse the progression of neurological deficits in RTT girls.

### Abstract

Rett is first manifested in 6-18-month-old children, correlating with a major period of postnatal neuronal maturation. With support from RSRF and IRSA, we have determined that newly matured MeCP2 mutant neurons have reduced density and an abnormal distribution of dendritic spines, confirming that MeCP2 is critical for neuronal maturation. However, most RTT patients are heterozygote females and are mosaic in MeCP2 deficiency due to random inactivation of the X chromosome. It is currently unknown whether the majority of neurons (54-80%) that express wild type (wt-Mecp2 expressing neurons) MeCP2 in heterozygote brains develop and function normally. Since inter-neuronal signaling has been shown to be critical for neuronal axon guidance, synaptic development, and functional pruning, it is possible that defective MeCP2 mutant neurons can negatively affect the maturation and function of wt-Mecp2 expressing neurons. This negative effect could be responsible for the progression of neurological deficits in RTT patients during both the initial onset of symptoms, and late stage deterioration. The goal of this proposal is to test the hypothesis that in heterozygote females, mutation of MeCP2 leads to altered inter-cellular signaling, which results in defective maturation and function in neurons expressing wild type MeCP2. With this goal, we propose to (1) characterize the maturation and function of neurons expressing wild type MeCP2 in an MeCP2 mosaic mouse model that contains wild type and mutant neurons readily distinguishable by GFP fluorescence; and (2) characterize the effects of Mecp2 null neurons on co-cultured wild type neurons in vitro. The outcome of this initial study will set the stage for further mechanistic investigations that will have significant implication in understanding the pathogenesis of RTT and therapeutic development.

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## Post-Doctoral Fellowship Awards

Megumi Adachi

University of Texas Southwestern Medical Center at Dallas

Mentor: Lisa Monteggia

Identification and characterization of MeCP2 target genes involved in synaptic transmission

\$100,000

### Lay Summary

RTT is primarily caused by mutations in the MECP2 gene. However, nothing is known about how the mutations in MECP2 lead to neurological disorders observed in RTT patients. MeCP2 is a protein that binds to DNA, resulting in repression of genes expression. The mutations in the MECP2 gene, which are predicted to result in the loss of MeCP2 function, have been suggested to cause a global imbalance of gene expression that may contribute to the neurological symptoms seen in RTT patients; yet, only a handful of genes have been proposed as target genes of MeCP2 that potentially relate to RTT. Our laboratory has recently found specific deficits in communication between neurons in the brain, called synaptic transmission, upon the loss of MeCP2 in the hippocampus, a region of the brain that plays an essential role in formation of learning and memory. Our data suggests that balanced gene expression is important in regulating neuronal communication in the brain and may provide insight into how the loss of MeCP2 function leads to the disease state. The goal of my proposal is to identify MeCP2 downstream target genes involved in synaptic transmission in hippocampal neurons. Once I identify these genes, I will investigate whether over-regulation of the identified genes in normal (wild type) neurons causes abnormal synaptic transmission similar to what we detected in hippocampal neurons lacking MeCP2. As a complementary approach, I will also examine whether restoring levels of these target genes in neurons lacking MeCP2, restores synaptic transmission to normal levels. These experiments will hopefully identify viable gene targets that when altered by the loss of MeCP2, contribute to functional deficits in neurons that may ultimately underlie aspects of RTT. This information is crucial in the development of effective therapeutic strategies for RTT patients.

### Abstract

Rett syndrome (RTT) results from mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) on the X chromosome. MeCP2 is thought to globally silence genes by binding methylated cytidine within the promoters of genes and then recruiting co-repressor protein complexes containing Sin3A and histone deacetylase. However, only a handful of genes have been proposed as target genes of MeCP2 that potentially contribute to pathology of RTT. Our laboratory has recently identified specific deficit in excitatory, but not inhibitory, synaptic transmission upon the loss of MeCP2 function in hippocampal neurons. Our data suggests that transcriptional repression is important in regulating presynaptic function in hippocampal neurons, and that MeCP2 is a critical regulator of this process. The goal of the proposed study is complement and extend our previous data by identifying target genes under MeCP2 control that are involved in synaptic transmission, and then testing whether alterations in levels of these target genes modify synaptic transmission in a MeCP2-dependent manner. Understanding the role of MeCP2 in presynaptic gene regulation as well as neurophysiological consequences of the dysregulated gene expression may provide important information on the molecular mechanisms underlying the pathophysiology of RTT.



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Ichiro Hiratani

SUNY

Mentor: David Gilbert

The effect of MeCP2 Deficiency on Epigenetic Regulation in Neuronal Lineages

\$100,000

## Lay Summary

RTT is a neurological disorder caused by mutations in a gene encoding MECP2. How the loss of MECP2 function leads to RTT is currently unknown. Because MECP2 exerts its function through binding to DNA, identification of its binding targets in the human genome (genome= a complete set of human genes) is essential to understand its function. Identification of genes bound and regulated by MECP2 would facilitate the development of new therapeutic paradigms that would circumvent the series of events caused by MECP2 malfunction in RTT at its earliest steps. However, targets of MECP2 are still unclear at present. Initially it was expected that MECP2 normally plays a role in shutting genes off. (One of the major roles of DNA binding proteins is to regulate the expression of genes, either to turn them on or off.). Thus, the assumption was that MECP2 target genes would be on in the absence of functional MECP2, which could lead to RTT. Despite such anticipation, studies have failed to detect major alterations in gene repression in MECP2-deficient mouse models. This may be because such studies relied only on changes in gene expression levels as readout to measure the effect of MECP2 deficiency. This underscores the need to develop different approaches to identify MECP2 targets. In this proposal, I will employ a novel approach for MECP2 target identification, focusing on its role in the regulation of "transcriptional competence" (a term to describe the potential activity of genes, or the likelihood of genes to be expressed) of its target genes, rather than gene expression per se. MECP2 binds to modified forms of DNA called methylated DNA. Low levels of DNA methylation correspond to high potential gene activity while high levels correspond to low potential gene activity. Thus, it is reasonable to assume a role for MECP2 in controlling "transcriptional competence" of its targets. To monitor "transcriptional competence", I will examine histone modifications and replication timing in mouse cells with or without MECP2. Both are recognized as important readouts of "transcriptional competence". This mouse whole genome will be analyzed. The remarkable similarity between the genomes of mouse and human allows the discoveries to be readily applied to human RTT etiology studies and therapeutic development.

## Abstract

Identifying the MECP2 binding targets is essential for understanding Rett Syndrome etiology and developing new therapeutic paradigms. However, MECP2 targets are still unclear at present. This may be because of the lack of focus on features other than transcription for MECP2 target identification. Thus, I propose to examine the epigenetic defects in MeCP2-deficient mouse cells during neural development using microarray approaches to identify MeCP2 targets. To monitor epigenetic states, I will perform genome-wide analyses of replication timing and histone modifications using microarrays. As a model system for neural development, I will employ a homogeneous neural differentiation system of mouse embryonic stem (ES) cells. DNA replication represents the time of de-nova assembly of all epigenetic components, hence the time and environment in which a gene is replicated is considered critical for either maintaining or re-modeling its epigenetic state. I recently verified that NimbleGen microarrays are remarkably suitable for replication timing analysis: over 90% of the 54 genes analyzed individually by PCR showed reproducibility on the microarray (Hiratani, unpublished). Microarray analysis of histone modifications by ChIP (Chromatin Immunoprecipitation)-chip assay has also become quite routine. Despite the increasing attention on epigenetic regulation and its importance, this is still a relatively unexplored area in Rett syndrome research. Together with the comprehensive nature of the project, the proposed research is highly promising and relevant to the goals of the RSRF.

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Denis Jugloff

Hospital for Sick Children

Mentor: James Eubanks

Serotonergic activity and seizure susceptibility in a mouse model of RTT \$100,000

## Lay Summary

One of the most common conditions co-existing in RTT is seizures. They are reported in approximately 50-80% of patients. Unfortunately, these seizures are often unresponsive to conventional anti-seizure drugs. Despite its common occurrence, very little information exists regarding why patients with RTT are so susceptible to seizures. Recently, I have performed some preliminary experiments on a mouse model of RTT which have produced rather intriguing findings - we now have new data to connect a brain chemical pathway known to play a key role in seizure generation, to the seizures observed in RTT. In this proposal, I outline experiments which build on this data, that range from basic science to initial therapeutic testing. These experiments will attempt to reveal the mechanisms underlying this newly discovered relationship, as well as attempt to correct this condition in a mouse model of RTT. As there has been limited benefit in current drug and diet strategies in treating the seizures in patients with RTT, any information which would shed light on the mechanisms underlying this common co-existing condition, or provide a new therapeutic strategy, would be of great value. Ultimately, my experiments will help us understand why seizures are so common in RTT and provide the initial proof-of-principle for the initiation of a novel drug-based interventional strategy to lessen the severity of this debilitating co-condition.

## Abstract

Despite the common occurrence of seizures in Rett syndrome, very little information exists regarding how the loss of MeCP2 affects seizure susceptibility. Interestingly, various studies have provided evidence to suggest that alterations in serotonergic activity may be of pathogenic importance in the Rett brain. Yet, the role of serotonin in seizure generation in Rett syndrome remains to be investigated. My preliminary data provide strong evidence suggesting measurable cortical dysfunction in the MeCP2-deficient mouse, and the involvement of the serotonergic system in seizure susceptibility: the administration of the non-specific 5-HT<sub>2</sub> agonist 4-iodo-2,5-dimethoxyphenylisopropylamine (DO1; (2 mg/kg, i.p.), induced pronounced slow spike and wave oscillations in MeCP2-deficient symptomatic mice. This activity was not blocked by the administration of diazepam, suggesting that altered GABA A behavior is not involved in these spike and wave oscillations. The mechanism underlying this 5-HT<sub>2</sub> agonist-induced epileptiform activity remain unidentified. Thus, the principal objective of this proposal to determine the involvement of serotonin (5-HT<sub>2</sub>) receptors in seizure generation in the MeCP2-deficient mice, through the examination of protein expression, pharmacological, electrophysiological, and behavioral parameters. Finally, I will provide proof-of-principle that subtype-specific 5-HT<sub>2</sub> antagonists may be therapeutically useful in Rett patients for ameliorating seizure activity. Based upon the available data and my preliminary results, I hypothesize that a specific 5-HT receptor subtype (5-HT 2A or 5-HT 2C) is responsible for agonist-induced seizure activity, and that application of the appropriate antagonist will be a therapeutic benefit in lowering the occurrence of spontaneous seizure episodes. My pilot studies are the first to link a potential synaptic pathway - known to play a key role in epileptic susceptibility - to seizures, a common co-morbidity of Rett syndrome. Indeed, as outlined above, these experiments will investigate the mechanism underlying heightened seizure susceptibility, as well as provide proof-of-principle for the use of a pharmacological-based intervention to attempt to lessen the severity of this debilitating co-morbidity of Rett syndrome.

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Alysson Muotri

Salk Institute

Mentor: Fred Gage

The contribution of MeCP2 to L1 retrotransposition and the generation of neuronal diversity: consequences for neuronal function and RTT phenotype

\$100,000

### Lay Summary

Brain formation is an incredibly wasteful process. About half of the nerve cells created in a developing brain have died by the time that brain has formed. Many researchers think that the cells that live and those that die is decided by a process similar to natural selection. Cells with the right properties in the right places flourish; those without wither. But natural selection requires random variation to generate the various properties. We recently found that L1 retrotransposons, the so-called "jumping genes" could provide that variation, by affecting gene expression depending on where they "jump" or land. We previously showed that MeCP2, the mutant protein in Rett and other neurodevelopmental diseases, is a negative regulator of L1 retrotransposons in the brain. Moreover, animal models resembling Rett patients display higher number of "jumps" in neurons when compared to normal (controls) animals. Such observation raised the possibility that part of the neurological symptoms of Rett patients may be caused by descontrolled L1 retrotransposition. The goal of this proposal is to characterize the role of MeCP2 in the control of "jumping genes" in nerve cells. For that purpose, we will use several rodent and human cells to monitor the activity of L1 retrotransposons in presence and absence of MeCP2, and the consequences of an attenuated L1 activity to a mouse model of Rett syndrome. The validation of this hypothesis will certainly open new potential possibilities for therapeutic interventions.

### Abstract

LINE-1 (L1) retrotransposons are active elements in the genome capable of mobilization in neuronal precursor cells, resulting in a mosaic brain (Muotri et al, 2005). Upon mobilization, LI insertions can alter gene expression, resulting in a genetic heterogenic population of neurons. However, the physiological consequence of somatic L1 retrotransposition is unknown. Our preliminary results showed that L1 retrotransposition levels are increased in a MeCP2 knockout mouse model, indicating that MeCP2 is important for LI repression during neuronal differentiation in a methylation-independent fashion. Based on this, we postulate that the phenotypic heterogeneity in RTT could be, at least in part, due to variable neuronal pattern of LI activity in the brain. The main objective of this proposal is to characterize the molecular complex that repress L1s in undifferentiated neural stem cells and the switch mechanism that unleash L1 expression during neuronal differentiation. Specific aims were designed to obtain results with cellular models (both rodents and humans) and to attenuate LI retrotransposition in MeCP2 knockout mice. A battery of behavioral tests will reveal if LI attenuation could rescue, at least partially, some of the neurological defects associated with this mouse model.

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Zilong Qiu

UCSD

Mentor: Anirvan Ghosh

The role of MeCP2 in dendritic growth and spine development on cortical neurons

\$100,000

### Lay Summary

One of the most remarkable features of the mammalian nervous system is that its development can be modified by both genetic programs and environment. Maturation of the mammalian brain takes more than ten years, starting from birth and continuing through puberty. Enrichment of positive stimuli can enhance neuronal function, while lack of stimuli may compromise normal development. RTT is a severe mental retardation disorder, occurring in 1 of 10,000 to 20,000 young girls. RTT patients exhibit nearly normal development for a short period after birth. Many of these patients start to lose cognitive and motor abilities between 6 to 18 months. Because of this delayed onset, the symptoms of RTT are thought to be caused by specific defects in the maturation, but not formation, of the central nervous system. 80-90% of RTT patients carry mutations in the MECP2 gene. The cognitive and motor defects found in RTT patients strongly suggest that a maturation process may be comprised in those patients. Dendrites are tree-like structures where a neuron receives and processes information coming from other neurons. Maturation of dendrites largely represents maturation of the neuron. Synapses are connections between neighboring neurons. The synapse is the basic functional unit through which neurons communicate with each other. Most neural activity underlying cognitive and motor activity is transmitted by synapses between different neurons. After being processed by synapses, neural activity in turn modifies the strength of synapses. In this proposal, I will examine whether and how loss of MeCP2 causes defects in the development of dendrites and synapses. After extensive characterization of the mechanism by which the development of dendrites and synapses is altered without MeCP2, I will explore the possibility that defects caused by loss of MeCP2 can be rescued by enhancing trophic and neuronal activity. Results from these studies could lead to new therapeutic strategies for treating RTT.

### Abstract

Rett syndrome (RTT; MIM 312750) is a severe X-linked dominant neurological disorder affecting young girls. Loss of function of methyl CpG bind protein 2 (MeCP2) is thought to be the predominant cause of Rett syndrome. The onset of Rett syndrome is associated with a progressive loss of cognitive and motor skills, which suggests a defect in neural connectivity. Consistent with this possibility, abnormal development of dendrites and synapses of cortical neurons has been reported in Rett syndrome patients. The overall goal of my project is to explore the possibility that mutations in MeCP2 lead to synaptic maturation defects that might be rescued by modulating BDNF or calcium signaling pathways. MeCP2 knockout mice express phenotypes similar to Rett syndrome patients. Thus, MeCP2 null mice are thought to be a reliable model to study the pathogenesis of Rett syndrome. The cognitive and motor defects in Rett patients may reflect a role for MeCP2 in dendritic and synaptic development, but this has not been thoroughly explored. I propose to characterize the defects in dendritic growth and synaptic maturation in MeCP2 null neurons. I will determine if the normal development of dendrites and spines is altered by the loss of MeCP2 in cultured cortical neurons. In order to explore possible ways to rescue defects caused by loss of MeCP2, I will investigate whether activation of BDNF and calcium signaling can bypass the requirement of MeCP2 in the development of dendrites and synapses. To accomplish this, I will enhance BDNF signaling pathway or apply specific glutamate receptor agonists to MeCP2 null neurons and examine whether these treatments can rescue the defects caused by the loss of MeCP2. Results from these studies could lead to new therapeutic strategies for treating Rett syndrome.

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