

## Research Awardees: G.E.A.R.

In January of 2004 RSRF announced a new fast track funding mechanism. The Grants of Excellence to Accelerate Rett Research (G.E.A.R.) are awarded at the discretion of the RSRF Board of Trustees to outstanding investigators who have shown a commitment to Rett Syndrome research. The aim of the G.E.A.R. is to accelerate ongoing research in key labs by injecting financial resources.

2006

Michael Greenberg , Ph.D.

Children's Hospital Boston, Harvard

Development of Novel Antibody Reagents for MeCP2

\$210,000

### Lay Summary

Mutation of the MECP2 gene has been found to result in the vast majority of Rett Syndrome (RTT) cases. Further insights into the basis of RTT, and the ultimate translation of this knowledge into improved patient outcomes, rely on the continued study of MeCP2 function at the protein level. However, RTT research continues to suffer from a relative lack of specific antibody reagents. We therefore propose to undertake the systematic development of antibody reagents for the MeCP2 protein that will be made available to the entire community of scientists carrying out research on RTT. These antibody reagents will allow for a detailed analysis of MeCP2 expression and subcellular localization as well as for biochemical study of MeCP2 interaction with other cellular components. In addition, we will go on to utilize these antibody reagents to characterize the interaction of MeCP2 with other cellular proteins. Thus, the proposed studies should provide the wider scientific community with access to improved reagents to the MeCP2 protein and may uncover new aspects of MeCP2 function in the nervous system that may ultimately provide new opportunities for the development of therapeutic strategies to alleviate RTT pathology.

2005

Juan Botas, Ph.D.

Baylor College of Medicine

Genetic approaches to identify compensatory mechanisms preventing or ameliorating Rett Syndrome pathogenesis

\$361,020

Research Sponsor: Gordon and Anne Rich/Reading Rock, Inc.

#### Lay Progress Report (August 2006)

Rett syndrome is a devastating neurological disorder affecting ~1 in 15,000 girls and leading to developmental regression and mental retardation. Most cases of Rett syndrome are caused by mutations in a gene called MeCP2. This gene works by preventing other "target" genes from functioning in the wrong time and place. Therefore, improper control of genes in the brain caused by MECP2 mutations is thought to cause Rett Syndrome. One possible avenue for therapy is to identify the MeCP2 target genes wrongly functioning during disease and to restore their normal control. An alternative approach is to identify molecular mechanisms capable of compensating for the improper control of target genes caused by MeCP2 mutations.

We are utilizing the fruit fly *Drosophila* as a novel in vivo system to identify components of the molecular machinery that functions antagonistically to MeCP2 and to alter its activity to compensate for MeCP2 loss of function. With this objective in mind, we generated transgenic *Drosophila* strains expressing human MECP2. We have already identified genetic modifiers in these animals expressing MeCP2 and we believe that they will lead to the identification of factors that are capable of compensating for MeCP2 lack of function. We will use mammalian cell systems to validate our findings in *Drosophila*, and to define possible therapeutic approaches.

#### Lay Summary

Rett Syndrome is a devastating neurological disorder affecting ~1 in 15,000 girls and leading to developmental regression and mental retardation. Most cases of Rett Syndrome are caused by mutations in a gene called MeCP2, and it is generally accepted that its role is to prevent other genes from functioning in the wrong time and place. This important discovery suggests that misregulation of genes in the brain caused by MECP2 mutations leads to Rett Syndrome. One possible avenue for therapy is to identify the MeCP2 target genes misregulated during disease and to restore their normal regulation. An alternative approach is to identify molecular mechanisms capable of compensating for the misregulation caused by MeCP2 mutants. We propose to use the fruit fly *Drosophila* as a novel in vivo system to identify components of the molecular machinery that functions antagonistically to MeCP2 and to alter its activity to compensate for MeCP2 loss of function. We have generated transgenic *Drosophila* strains expressing either human MECP2 that will be used towards this objective. We hypothesize that screening for genetic modifies in the MeCP2 flies will lead to the identification of factors that are capable of compensating for MeCP2 lack of function. Once these factors are identified in *Drosophila*, further work will be carried out in mammalian cells to validate our findings, and to define possible therapeutic approaches.

#### Abstract

Rett Syndrome is a neurodevelopmental disorder affecting ~1 in 15,000 girls and leading to developmental regression and mental retardation. The discovery that most cases of Rett Syndrome are caused by mutations in the MeCP2 transcriptional repressor suggests that misregulation of neuronal genes caused by MECP2 loss of function leads to Rett Syndrome pathogenesis. One possible avenue for therapy is to identify the MeCP2

target genes misregulated during disease and to restore normal regulation. An alternative approach is to identify molecular mechanisms capable of compensating for the misregulation caused by MeCP2 loss of function. We propose to use *Drosophila* as a novel in vivo system to identify other components of the chromatin remodeling machinery that function antagonistically to MeCP2 and to alter their activity/activities to compensate for MeCP2 loss of function. We have generated transgenic *Drosophila* strains expressing either wild type or mutant human MECP2 that will be used towards this objective. We hypothesize that screening for genetic modifiers in the MeCP2 flies will lead to the identification of factors that are capable of compensating for MeCP2 lack of function; e.g., components of the chromatin remodeling machinery. Other MeCP2 genetic modifiers will identify genes modulating MeCP2 levels or activity through affecting its stability or modifications.

[Back to Top](#)

Richard Goodman, M.D., Ph.D.

Vollum Institute, OHSU

Regulation of MeCP2 by CREB-induced microRNAs

\$220,000

### Lay Summary

Mutations on the MeCP2 gene are responsible for most instances of Rett Syndrome. Elucidating how MeCP2 contributes to normal brain function is essential for understanding how its loss causes abnormalities in patients. The MeCP2 RNA is unusual in that it contains an extremely long flanking region called the 3' UTR. This region does not code for the MeCP2 protein, but rather provides signals that determine how much protein is made. We have discovered an entirely unexpected aspect of MeCP2 regulation - that the production of the protein is regulated by small noncoding RNAs termed microRNAs. We have evidence that the production of these microRNAs is controlled by neuronal activity. Our studies will explain how MeCP2 function is regulated over the long term, which is particularly important for its effects on neuronal maturation.

### Abstract

Our underlying hypothesis is that MeCP2 translation is controlled by a family of microRNAs that interact with specific target sequences in the MeCP2 3' UTR. These microRNAs were identified by virtue of their regulation by the transcription factor, CREB. Thus, we propose that CREB induces the synthesis of a family of microRNA molecules that

regulate MeCP2 production. This pathway may provide a mechanism for the long-term regulation of MeCP2 function and extends previous models. Our specific aims are to characterize the mechanism of the translational arrest, identify pathways that induce the microRNAs, and determine how these pathways are influenced by development.

[Back to Top](#)

2004

Huda Zoghbi, M.D. and Nathaniel Heintz, Ph.D.

Baylor College of Medicine and Rockefeller University

Novel Strategy for Identification of Neuron-Specific MeCP2 Targets

\$522,504

#### Lay Summary

Rett Syndrome is a complex disorder characterized by diverse neurological problems that can be traced to different types of neurons. Because the Rett syndrome gene encodes a protein that normally regulates the expression of other genes, we propose that the various features of Rett syndrome result from abnormal expression of genes in various types of neurons. The complexity of the nervous systems (thousands of different neuronal types and a high percentage of supporting cells compared to neurons ~ 10:1), makes finding neuron-specific changes very challenging and requires that we develop a sensitive strategy to capture neuron-specific gene changes in mouse models of Rett syndrome. We have developed a strategy, termed "BACarrays" that will permit us to purify the RNAs from specific neurons using a tag that is expressed in these neurons. To do this we will express a protein tag that can bind cellular RNAs in the neurons of interest. When we isolate this protein tag we will capture the RNAs expressed in these specific neurons. We will use this strategy to study the patterns of gene expression in five different types of neurons. These specific neuronal types have been selected because of the clinical features commonly seen in Rett syndrome. Once we identify neuron-specific changes in gene expression we can begin to address questions regarding the mechanism by which MeCP2 causes specific neuronal abnormalities and identify pathways that potentially may be targeted pharmacologically.

#### Abstract

The overall goal of this project is to identify neuron-specific targets of MeCP2 in order to elucidate the molecular basis of the various clinical features of Rett syndrome. MeCP2 is a transcriptional repressor thus it is very likely that many of the Rett phenotypes are secondary to altered neuronal gene expression. We have generated a mouse model of Rett syndrome (Mecp2 308 allele) that reproduces many features of the human disease. To identify MeCP2 targets that mediate the specific phenotypes we plan to generate BACarray transgenic lines that express EGFP-tagged ribosomal protein in different types of neurons. BACarray lines will be generated for presynaptic and postsynaptic dopaminergic neurons, for serotonergic neurons, and for hypothalamic neurons. Male transgenic mice from these lines will be crossed to heterozygote Mecp2 308/+ females and the mRNAs (which normally assemble into polysomes) will be purified, using high affinity anti-EGFP6 antibody, from wild-type and Mecp2 308/Y animals of different ages for comparative gene expression profiling. mRNA expression changes that differ between wild-type and Mecp2 308/Y mice will be identified and the expression differences will be confirmed for a subset of the targets. This approach will provide us for the first time with data sets on gene expression changes that are cell-specific, expression changes common to all neurons, as well as expression changes that temporally correlate with the phenotype. Having these data we can then begin to probe the molecular mechanism mediating the Rett syndrome phenotype.

[Back to Top](#)

Adrian Bird

G.E.A.R. Award Lay Summary

About 80% of Rett Syndrome patients possess a new mutation in the MECP2 gene. Disruption of the equivalent Mecp2 gene in mice causes symptoms that resemble aspects of human Rett Syndrome. We will make use of this model system to ask basic questions about the behavior of the MeCP2 protein in living cells and about the consequences of its loss from brain cells.

By labeling the MeCP2 protein with a fluorescent tag, we will follow its movements in the nerve cell nucleus using sensitive microscopy techniques. This will tell us how tightly the protein is bound to chromosomes. It will also allow us to ask how these dynamic properties are affected by nerve cell activity and by Mecp2 mutations like those found in Rett patients.

In addition, we will try to relieve the symptoms of

MeCP2 deficiency by activating an Mecp2 gene in the mutant mice. This will be done by reversing a block on the Mecp2 gene once symptoms have appeared, or by artificially administering a synthetic Mecp2 gene. In this way we hope to determine whether Rett-like symptoms in this model system can be rescued if normal MeCP2 protein is restored.

Links:

[www.wcb.ed.ac.uk/bird.htm](http://www.wcb.ed.ac.uk/bird.htm)

[www.wellcome.ac.uk/en/1/awtviswhogov.html#Bird](http://www.wellcome.ac.uk/en/1/awtviswhogov.html#Bird)

[Back to Top](#)

Rudolf Jaenisch

Whitehead Institute

A Mouse Model for Rett Syndrome

\$259,568

G.E.A.R. Award Lay Summary

My laboratory uses mouse genetics to understand the molecular basis and the pathology of Rett syndrome. We use engineered mice and genomic approaches as tools to define the molecular targets of Mecp2 and to devise strategies that can influence the severity of symptoms in Mecp2 mutant mice. Our goal is to devise molecular paradigms that eventually could help to alter the manifestation or progression of Rett syndrome.

We will use the RSRF funds for two different aspects of our program.

1. One of the most important and as yet unresolved issues in the pathoetiology of RTT is whether the disease is caused by the abnormal function of postnatal neurons at a time when symptoms become apparent in affected girls or, alternatively, whether it is a developmental disorder with postnatal manifestation in the CNS. Thus, the key question is whether mutant neurons are normal at early postnatal ages and become dysfunctional only later. Our approach to this question is to generate various transgenic mouse strains that allow inducible expression of Mecp2 in mutant mice of different ages. We are interested to investigate whether the restoration of normal Mecp2 expression in a

mutant mouse would affect the incidence or progression of the disease.

2. Of crucial importance for understanding the consequences of Mecp2 deficiency is the identification of target genes whose activity is affected by the protein. Recently, genomic tools such as expression chips that are essential for molecular target identification have become available. We are collaborating with the laboratory of Richard Young at the Whitehead Institute to build a platform that will allow us to conduct genome wide search for genes whose activity is regulated by Mecp2.

The Grant of Excellence for Accelerated Rett Research comes at a time of a tight NIH budget and will allow us to concentrate on aspects of our project that would otherwise be delayed because funds would need to be secured from other sources.

Links:

[www.wi.mit.edu](http://www.wi.mit.edu) (1)

[www.wi.mit.edu](http://www.wi.mit.edu) (2)

[www.wi.mit.edu](http://www.wi.mit.edu) (3)

[Back to Top](#)

Huda Zoghbi

G.E.A.R. Award Lay Summary

Pre-Clinical Interventional Trials in Mecp2308 Mice

Behavioral and molecular studies of a mouse model of Rett syndrome (Mecp2308 allele) are beginning to reveal features and pathways that can be pharmacologically treated or modulated. For example, anxiety is a prominent and reproducible phenotype in the Mecp2308/Y mice and is reminiscent of the increased anxiety, screaming, and sometimes hyperventilation seen in the Rett girls when faced with a new situation. Having established a way to evaluate and quantify the anxiety phenotype in the Mecp2308/Y mice, we would like to begin pharmacologic interventions to see if we can decrease the anxiety or eliminate it with such treatments. We also plan to test if the reduced anxiety has a beneficial effect over other phenotypes such as social behavior abnormalities and the presence of stereotypies. We would like to try the treatment with symptomatic mice and if we find benefit, proceed to treat pre-symptomatic mice to see if reducing anxiety will reduce the burden of the disease. Initial treatment

protocols will be acute and short-term with benzodiazepines followed by chronic treatment protocols using drugs that enhance levels of serotonin.

Another prominent feature of the Mecp2308 mice is the tremor, which incidentally increases significantly in anxiety-provoking situations. We will investigate the effects of beta-blockers such as propranolol on these tremors as well as on the anxiety phenotype. Before we embark on the propranolol trial, we will establish protocols to quantify the tremors so that we can be sure about the effects of therapeutic intervention. The quantifiable outcome measures we will develop for the motor phenotypes, together with the ones already developed for the behavioral features, will prove useful for additional therapeutic trials in the future.

As we continue to define phenotypes and molecular changes with the Mecp2308/Y mice, we will be in an excellent position to investigate additional pharmacologic treatments and to determine if combinational pharmacologic therapy is superior to single agent therapy.

It is our hope that treatments that prove effective and safe with this pre-clinical setting can then be implemented through multi-center clinical trials in patients.

Links:

[www.imgen.bcm.tmc.edu](http://www.imgen.bcm.tmc.edu)

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

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

[Back to Top](#)