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Research Awards

Laura Carrel, Ph.D.

Case Western Reserve University

"Expression Profile of the Inactive X Chromosome in Rett Syndrome: Role of MeCP2 in the establishment and maintenance of X inactivation."

2-Year Award: \$95,790

Research Sponsor: Family & Friends of Jessie Lebson

Final Report:

Because the methyl-binding protein MeCP2 plays a role in regulating gene expression, Rett Syndrome is likely caused by misregulation of genes normally silenced by MeCP2. To date, the identity of such genes remains unknown. As methylation is involved normally in silencing genes on the inactive X chromosome, it is plausible that genes on the X are regulated by MeCP2 and therefore may be inappropriately expressed in cell lines from Rett syndrome patients. To test this hypothesis, we isolated the cell lines necessary for these experiments and assayed a number of X-linked genes. For all genes tested, reactivation was not observed in the Rett cell lines. Additionally, we looked for methylation changes at four X-linked genes, but also saw no significant differences between cell lines derived from Rett Syndrome individuals and normal controls. These studies argue that MeCP2 is either not involved in silencing the genes tested, or at a minimum, that it does not play an essential role in silencing these genes, at least in the cell lines examined. Assays have been developed that can be used to test additional genes on the X. While these studies could identify candidate genes that may be responsible for clinical features of Rett Syndrome, they may also help us to determine whether MeCP2 plays a role in silencing X-linked genes. However, as minimal changes have been observed in the cell lines

examined, it may be more important to focus future studies on examining X-linked gene expression in the neuronal tissues that are affected in Rett Syndrome individuals by using the animal models that have been developed.

Lay Summary

Mutations in the MECP2 gene have been recently shown to be responsible for Rett Syndrome. The MeCP2 protein is involved in regulating gene expression and therefore, the clinical features of Rett Syndrome are likely caused by misregulation of genes that are normally silenced by MECP2. However, to date, the identity of such genes remains unknown. The goal of this project will be to analyze a large number of genes on the X chromosome and determine whether any of these genes are inappropriately expressed in cell lines from Rett Syndrome patients. If abnormal gene expression is observed, we will determine whether these genes can be properly controlled if an intact version of the protein is introduced into cells. These studies will be important for identifying which candidate genes may be responsible for clinical features of Rett Syndrome, for understanding the basic biology question of what role MeCP2 plays in silencing X-linked genes, and for determining whether introduction of the MECP2 gene into cell lines could re-establish appropriate gene silencing, a critical question if therapeutic approaches for Rett Syndrome are considered.

Abstract

Rett Syndrome (RTT) is caused by mutations in the X-linked gene, MECP2, a methyl-cytosine binding protein involved in transcriptional repression. This suggests that RTT phenotypes reflect at least partial derepression of normally silenced genes. Target genes are unknown, but plausible candidates include genes on the X chromosome that are epigenetically silenced by X inactivation. The objective of this project is to determine whether X-linked gene expression is altered in RTT. Genes on the inactive X have features consistent with repression by MeCP2, yet whether this protein is involved in any aspects of X inactivation remains untested. X inactivation patterns in most RTT females are random establishing that MeCP2 is not essential for X inactivation. Nonetheless, replication timing of some chromosome bands on the inactive X may be altered in RTT consistent with abnormal gene silencing. We propose that MeCP2 may be necessary for silencing only a subset of X-linked genes. Therefore, to determine whether inactive X gene expression is altered in RTT warrants an approach geared towards analyzing a large number of X-linked genes. The experiments in this project have two aims: to create an X inactivation profile of the inactive X in RTT by evaluating 100 X-linked expressed single nucleotide polymorphisms (SNPs) and determining the inactivation state of all informative SNPs in a panel of cell lines derived from RTT females and, to test whether introduction of an intact copy of MECP2 into cell lines derived from females with RTT alters the inactivation state of any of these X-linked genes. These studies will be important for identifying candidate genes that may be responsible for RTT phenotypes, for understanding the role that MeCP2 plays in silencing X-linked genes, and for determining whether introduction of the MECP2 gene into RTT cell lines could re-establish appropriate gene silencing, a critical question if therapeutic approaches for RTT are considered.

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Rudolf Jaenisch, M.D.

Whitehead Institute for Biomedical Research and M.I.T.

"A Mouse Model for Rett Syndrome"

2-Year Award: \$259,568

Research Sponsor: The Festival of Food and Wine

Lay Progress Report:

The identification of mutations in the X chromosomal MECP2 gene as the genetic defects underlying the development of Rett Syndrome presents us with a good opportunity to study the pathogenesis of the disease at the molecular level. The mouse Mecp2 gene is highly similar to the human gene and studies of the mouse gene may, therefore, help establish an animal model for Rett Syndrome. Using the gene targeting strategy, we have recently generated mice that lack Mecp2 either in all tissues or selectively in the brain. The mutant mice show features that are similar to those in Rett Syndrome patients, including apparent normal pre and perinatal development but fast postnatal deterioration. We have designed experiments to further analyze these Mecp2 mutant mice, focusing on revealing any cellular defects associated with Mecp2 deficiency in the mouse central nervous system. We are also conducting studies to determine any potential behavioral defects in the mutant mice. We hope that the results of these studies will not only provide insights into the function of the MECP2 gene and how a disease may arise when the gene is defective, but also lay a foundation for designing potential strategies to cure the disease.

Lay Summary

The identification of mutations in the X chromosome MECP2 gene as the genetic defects underlying the development of Rett Syndrome patients presents us with a good opportunity to study the pathogenesis of the disease at the molecular level. The mouse MECP2 gene is highly similar to the human gene and studies of the mouse gene may, therefore, help establish an animal model for Rett Syndrome. Using the gene targeting strategy, the applicants have recently generated mice that lack MECP2 either in all tissues or selectively in the brain. The mutant mice show features that are similar to those in Rett Syndrome patients, including apparent normal pre- and perinatal development but fast postnatal deterioration. In the project we plan to carry out well-designed experiments to further analyze these MECP2 mutant mice, focusing on revealing any cellular defects associated with MECP2 deficiency in the mouse central nervous system. The applicants also plan to conduct studies to determine any potential behavioral defects in the mutant mice. The applicants believe that the results of their studies will not only provide insights into the function of the MECP2 gene and how a disease may arise when the gene is defective, but also lay a foundation for designing potential strategies to cure the disease.

Abstract

We intend to establish a mouse genetic model for the human Rett Syndrome. Using the cre/lox conditional targeting strategy, the applicants have recently generated mice carrying a conditional allele of the mouse MECP2 gene. Through the use of transgenic mice carrying various cre recombinase transgenes, we have also obtained mice in which the MECP2 gene is inactivated either in all tissues (MECP2-null) or selectively in all regions of the brain (MECP2 brain-specific mutants). Surprisingly, both lines of mutant mice are viable and grossly normal at birth, and remain healthy for 3 to 4 weeks. Beginning at around 5 weeks of age, however, both mutant mice start to show abnormal features that are similar to those observed in Rett Syndrome patients, including nervousness, less physical movement, body trembling, and gait ataxia. These initial observations suggest that the MECP2 mutant mice may represent an excellent experimental system to study the pathogenesis of Rett Syndrome. We plan to further analyze the MECP2 mutant mice, with the focus on revealing any cellular defects associated with MECP2 deficiency in the central nervous system. Given the documented role of MECP2 in gene expression regulation, we will apply the Affymetrix gene chip technology to determine changes in gene expression in the mutant brain tissues. We also plan to conduct studies to determine potential behavioral defects in the mutant mice. Finally, we will introduce a mutated DNA methyltransferase gene into the MECP2 mutant mice to study the combined effects of both DNA hypomethylation and MECP2 deficiency on the developmental and function of the nervous system.

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Janine M. LaSalle, Ph.D.

University of California, Davis

"The Role of MECP2 in Parental Imprinting"

2-Year Award: \$99,522

Lay Progress Report (August 2002)

Rett syndrome is caused by mutations in the MECP2 gene on the X chromosome. Females with RTT have two X chromosomes, one with the mutant and one with the normal MECP2. Because of X inactivation in females, a process in which one X chromosome is randomly inactivated in each cell, RTT girls are mosaic for cells containing either the mutant or normal MeCP2 protein. We have used two different approaches to identifying cells with mutant MeCP2 in blood and brain of female RTT patients. From blood, the technique of single cell cloning was performed in order to separate cells containing mutant and normal MeCP2 proteins. T cell clones with mutant MeCP2 were isolated from four different RTT patients, but were always less frequent than clones with normal MeCP2. These results demonstrate that MECP2 mutations adversely affect lymphocyte growth. No difference was observed between normal and mutant cells in the expression of four different genes regulated by methylation or parental imprinting. For detection of mutant and normal MeCP2 in brain, we have used the novel technology of laser scanning cytometry to quantitatively detect the normal but not mutant MeCP2 protein. Brain cells with mutant MeCP2 were randomly distributed. Surprisingly, there were two different populations of cells based on amount of MeCP2 in normal brain samples. The elevated expression of MeCP2 was demonstrated to be unique to tissues in the central nervous system, providing a potential explanation for the specific neurologic symptoms in RTT. These results are expected to be important in understanding the role of MeCP2 in the pathogenesis of RTT and in designing therapies.

Lay Summary

Rett Syndrome is caused by mutations in the MECP2 gene on the X chromosome. Females with RTT have two X chromosomes, one with the mutant and one with the normal MECP2. Because of X inactivation in females, a process in which one X chromosome is randomly inactivated in each cell, RTT girls are mosaic for cells containing either the mutant or normal MeCP2 protein. In this project, the technique of single cell cloning will be performed on blood samples from RTT girls in order to separate cells containing mutant and normal MeCP2 proteins. These single cell clones will then be tested for changes in expression and methylation of two imprinted genes. Parental imprinting is a process in which a gene obtains a distinct maternal or paternal methylation "imprint" that allows one parental copy of the gene to be silenced. Since MECP2 is known to regulate the silencing of methylated genes, we hypothesize that cells containing mutant MECP2 may lose the methylation imprint and reactivate expression on the maternal chromosome.

Abstract

The discovery of mutations in the MECP2 gene in patients with Rett Syndrome (RTT) has broadened the role of epigenetic gene regulation in human development. MECP2 selectively binds to methylated CpG residues and resides in the transcriptionally inactive heterochromatic regions of the nucleus. Genomic imprinting is a process by which epigenetic differences between parental chromosomal alleles are obtained in the gametes. Silencing of imprinted gene expression has been hypothesized to be due to allelic differences in DNA methylation that can be detected for most imprinted genes. Therefore, the hypothesis of this proposal is that MECP2 is involved in silencing imprinted gene expression through the recognition of allele-specific methylation. The hypothesis predicts that cells from RTT patients with MECP2 mutations will show biallelic rather than monoallelic expression of imprinted genes. RTT is a dominant X-linked disease affecting heterozygous females. RTT lymphocytes exhibit random X inactivation, meaning the MECP2 mutations are only observed in RNA transcripts from approximately half the cells. We plan to use the novel approach of single T cell cloning from RTT blood so that clones differentially expressing wild type (wt) and mutant MECP2 could be examined in the same individual. The role of MECP2 in silencing imprinted gene expression and maintaining allele-specific methylation will be directly determined in these T cell clones. The results of the proposed experiments are expected to be directly relevant to understanding how mutations in MECP2 cause RTT by directly investigating genes that are normally silenced through methylation.

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Jonathan Pevsner, Ph.D.

Kennedy Krieger Institute

"Gene Expression in MECP2-Null Brain and Human Rett Syndrome Brain"

1-Year Award: \$50,000

Lay Summary

Rett Syndrome (RTT) is caused by mutations in the MECP2 gene. This gene binds to DNA and controls the ability of many other genes to be expressed (turned on). We have created a mouse lacking this gene. Male mice appear to have behavioral problems that are reminiscent of the human disease. In previous studies, we measured the expression levels of thousands of genes in the postmortem brains RTT versus normal girls. We propose to study gene expression in the mouse model. By comparing human and mouse results, we may use the advantages of each system to learn what changes occur in the brain that could cause the disease. We will also compare studies of small pieces of brain to studies of gene expression in single, identified brain cells.

Abstract

Rett Syndrome (RTT) is associated with mutations in the MECP2 gene, and mutations in this gene have now been identified in approximately 80% of patients. MeCP2 is a nuclear protein that binds to methylated sites in genomic DNA and can cause transcriptional silencing of nearby genes. RTT may therefore be caused by failure to read the DNA methylation signal appropriately. In recent studies we have introduced a deletable version of the MECP2 gene into the wild-type MECP2 locus. Preliminary phenotypic analyses suggest that the MECP2-null mouse is a model for aspects of RTT. In other studies we have measured gene expression in human postmortem RTT brains using high-density cDNA microarrays. A key question is why mutations in MECP2 may cause the selective neurological phenotype of RTT. We will integrate gene expression studies of human brain and single cells with studies of the mouse model brain and single cells. The specific aims are (1) Measure the expression of 8,700 genes in the brain of MECP2-null male mice relative to matched controls, (2) measure gene expression on microarrays using single cells isolated from the mouse brain, and (3) measure gene expression on microarrays using single cells isolated from the human RTT brain. In each case, we will confirm the abnormal expression of several genes by Northern blotting and in situ hybridization. Bioinformatic analyses will be used to compare gene expression findings between RTT patients and the mouse model for RTT, and to compare single cell findings to results obtained from whole brain.

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Jan Marino Ramirez, Ph.D.

University of Chicago

"Neural Mechanisms Underlying Erratic Breathing"

2-Year Award: \$78,960

Lay Progress Report:

Increasing evidence indicates that Rett Syndrome children are deficient in Substance P (SP) in brainstem areas that are associated with the central control of breathing. We use an animal model to investigate the consequences of Substance P deficiency and the role of substance P in controlling the regularity of breathing.

In the first funding period we concentrated on investigating two issues: (1) how does substance P regulate the frequency and regularity of respiratory network activity and (2) which intracellular, second messenger pathways mediate the substance P effect. Understanding both, the network and intracellular mechanisms, is important for the future development of a rationale therapy for erratic breathing. Our investigations yielded two unexpected results: (1) The regularity and the frequency of respiration depends on synaptic interactions between neurons with pacemaker and non-pacemaker properties. This interaction is controlled by both NMDA and GABAergic synaptic mechanisms. Enhancing NMDA and GABAergic synaptic mechanisms suppresses pacemaker properties and enhances the synchronization between non-pacemaker respiratory neurons, which then results in an increased regularity of breathing and decreased frequency of breathing. We currently investigate the hypothesis that deficiency in substance P leads to a decreased activation of NMDA and GABAergic mechanisms, which then result in erratic breathing. (2) A second set of experiments examined which second messenger pathways are involved in the substance P modulation of breathing.

We can demonstrate that the phospholipase D pathway is involved in the SP effect on sigh activity. However, despite examining all major second messenger pathways, we were unable to identify a second messenger system that mediates the SP effect on eupneic (normal, regular breathing) activity. Therefore, we currently test the hypothesis that the SP effect on eupneic activity is mediated by a membrane-delimited G-protein modulation and not by a cytoplasmic second messenger system.

We predict that understanding how substance P deficiency within the CNS leads to an irregularity of breathing is an important first step to treat erratic breathing. In the second funding period we will continue to unravel the underlying cellular mechanisms.

Lay Summary

We propose to develop a rational therapy for the treatment of erratic breathing in Rett Syndrome. It is well established that Rett Syndrome children are deficient in Substance P in brainstem areas that are associated with the central control of breathing. In this project we will use an animal model, which mimics this very characteristic brain pathology: i.e. a mutant mouse, which is Substance P deficient. These SP deficient mice generate erratic respiratory rhythmic activity, which resembles the situation in Rett Syndrome. The use of these mutated mice enables us to investigate in depth the neural mechanism that lead to erratic breathing and will allow us to develop substances that increase the regularity of breathing. Our investigations will cover all levels of integration from the molecular level to the level of the respiratory network. We predict that this proposal will enable us to propose a set of substances, which will be useful to increase the stability of breathing and treat erratic respiratory activity.

Abstract

We will investigate in an animal model the neural mechanisms that underlie erratic breathing, which is one very devastating symptom of Rett Syndrome (RTT). Because evidence indicates that, in RTT patients, the brainstem areas associated with the central control of breathing are deficient in Substance P, we will specifically examine the hypothesis that the irregular breathing in RTT is due to brainstem deficiency of Substance P. We hypothesize that an understanding of how Substance P regulates breathing will be essential for developing rational therapies for breathing disorders in RTT. We aim to provide an in-depth characterization of the role of Substance P in the neuronal control of breathing, covering the entire range from the membrane and molecular level to the synaptic and network level in both wildtype and Substance P knock-out (PPTAkO) mice. All proposed research will be carried out in a transverse slice preparation that isolates the neuronal breathing center. We have the following aims to test three specific hypotheses. Aim 1 investigates whether Substance P regulates the respiratory frequency by modulating pacemaker neurons. Aim 2 investigates whether Substance P increases the regularity of respiration by modulating synaptic inhibition. Aim 3 investigates whether irregular respiration can be stabilized by drugs acting on the mechanisms that mediate the response to

Substance P. These hypotheses are based on our preliminary results indicating that irregular respiration can be reversed either by application of Substance P or by antagonizing synaptic inhibition. We expect that it will be possible to stabilize breathing not only by Substance P, but also by any other drug that acts on the same neural pathway from the Substance P receptor to the electrical coupling between pacemakers and follower neurons. Understanding the neural mechanisms that control the regularity and stability of the respiratory rhythm and finding substances that increase the regularity and stability of respiratory activity will be a first important step to develop a rationale therapy for treating erratic breathing in Rett Syndrome.

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Paul Wade, Ph.D.

Emory University

"Methylated DNA binding proteins: Structure, function, and molecular interactions"

2-Year Award: \$100,000

Research Sponsor for Both Years: The Massachusetts Rett Syndrome Association

Final Report (December 2002):

Specific Aim 1 proposed to create novel, improved reagents for the study of MeCP2 and other members of the MBD family. We have embarked on the production of monoclonal antibodies to full-length human MeCP2. Three mice were immunized and subsequently boosted three times. All three mice exhibited very high titer antibodies against MeCP2 as measured by ELISA. We performed two separate fusions to produce hybridoma lines. Following the first fusion, our screening and subcloning process resulted in the isolation of four clonal lines that produce antibodies against human MeCP2. Preliminary epitope mapping demonstrates that the epitopes for two of these antibodies are in the far carboxyl terminus of the protein \sim beyond amino acid 294. The epitopes for the remaining two antibodies lie within the first 168 amino acids of the protein. We thus have two C-terminal antibodies and two N-terminal ones. In addition, the second fusion yielded 3 additional hybridomas that are TRD specific.

Our evaluation of these antibodies determined that hybridoma supernatants were too dilute to be useful for immunofluorescence or immunoblotting. We plan to produce ascites in mice to obtain more concentrated antibodies.

Specific Aim 2 proposed to characterize the consequences of a subset of Rett Syndrome mutations in MeCP2 to its structure, stability and function.

We have generated 15 missense mutations in the MBD domain of MeCP2. These mutants can be expressed as full-length recombinant protein in bacteria, as MBD domain recombinant protein in bacteria, or in mammalian cells with a FLAG epitope tag. We are currently systematically determining DNA binding properties, structural consequences of mutation, stability, cellular localization, and association with Sin3A and HDAC1 for each of these mutants.

We have used the preliminary data assembled during the RSRF funding period into an RO1 proposal for NIH. This grant application was submitted in February, and received good scores just out of the fundable range. We have resubmitted a revised application which is currently pending. If this proposal is funded by NIH, we will continue with the studies

initiated during our RSRF funding period.

Lay Summary

The MeCP2 protein is a member of a family of related proteins that bind to specifically modified regions in chromosome. Genetic changes in MeCP2 cause Rett Syndrome, a developmental disease. This research seeks to develop new methods and reagents for the study of MeCP2 and proteins closely related to it. These new reagents and methods will be used to determine how genetic changes in MeCP2 affect its properties and its function. In addition, we will apply these reagents and methods to the study of the larger family of proteins of which MeCP2 is a single member.

Abstract

Modification of DNA in eukaryotic cells by methylation of cytosine residues within CpG dinucleotides leads to the assembly of specialized chromatic architecture. Methylated regions of the genome are insensitive to common structural probes and genes located within them are transcriptionally repressed. A family of proteins with a conserved DNA binding motif termed a methylated DNA binding domain, or MBD, is responsible for recognizing the regulatory information inherent to DNA methylation and translating it into specialized functional states. Mutations in the prototype MBD family member, MeCP2 are causal for a majority of the cases of a developmental neurological disorder, Rett Syndrome. The objectives of this research are to develop experimental approaches for investigating structural consequences of the Rett Syndrome mutations in MeCP2, to understand the consequences of these mutations to MeCP2 function, and to develop reagents and assay systems applicable to the broader study of the MBD protein family. Specifically, we will generate panels of monoclonal antibodies against MeCP2 and the MBD protein family. We will apply these reagents to the study of the entire MBD family in order to define cellular and nuclear localization patterns, to determine abundance and association with other proteins, and to initiate the study of partitioning of these proteins in the genome. In conjunction with these studies, we will use purified recombinant protein to determine the domain structure of MeCP2, to analyze global structural alterations resulting from Rett mutations, and to determine their DNA binding properties. We will correlate the finding of these in vitro structural studies with in vivo, functional data on wild type and mutant MeCP2.

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Post-Doctoral Fellowship Awards

Brian Chadwick, Ph.D.

Case Western Reserve University

"Heterochromatin Protein Localization and Effect on Gene Silencing in Rett Syndrome"

Mentor: Huntington Willard, Ph.D.

2-Year Award: \$100,000

Research Sponsor: Reading Rock, Inc.

Lay Final Report (September 2002):

During development, cells differentiate into a wide range of highly specialized cell types such as neurons in the brain. Coupled with these changes are specific alterations in the regimen of genes expressed. Maintaining such changes is critical for the cells maturation and function. The past decade has seen massive advances in our understanding of the regulation of gene expression. Of particular interest is the research into the family of methyl-DNA binding proteins that are implicated in repressing gene expression. Defects in one member of this group MECP2, is the molecular basis of Rett syndrome. It is thought that MECP2 mutations in Rett syndrome patients compromises the ability of MECP2 to function and consequently results in inappropriate gene expression. To investigate the effects of MECP2 mutation in Rett syndrome, we chose a cell culture based approach. We anticipated that mutations in MECP2 would not only affect its nuclear distribution, but would also affect a variety of other proteins involved in the silencing process. In order to proceed with our investigation, several technical considerations were accomplished, including immortalizing and separating mutant cells from normal cells. We determined conditions to investigate a large number of components involved in gene regulation, and made comparisons between the mutant and normal cell lines. Surprisingly, other than MECP2 itself, no distribution changes were found with any of the proteins investigated including factors known to interact with MECP2. From this we conclude that while in most cases MECP2's role is redundant, likely only a handful of sites in the nucleus are absolutely dependent upon the function of MECP2. Homing in on these regions and monitoring the changes brought about by mutant MECP2 will allow more focused investigation into the devastating pathology in Rett syndrome.

Abstract

The molecular basis of Rett Syndrome (RTT) has recently been identified as mutations in the MECP2 gene. MECP2 is a transcriptional repressor that binds to methylated DNA and recruits protein complexes that alter the local chromatin environment and silence gene expression. It is likely that mutations of MECP2 impair its ability to either bind methylated DNA or recruit transcriptional repressor complexes. We hypothesize that as a consequence, a large number of genes that would normally be transcriptionally silent are active, causing the devastating effects observed in patients with RTT.

In female animals in order to achieve comparable levels of X-linked gene expression in males and females, one of the two X chromosomes is inactivated and can be readily identified in the nucleus by virtue of its characteristic heterochromatic state (Barr body). We propose to investigate the nuclear localization of a large number of known and novel heterochromatin proteins in RTT and control cell lines using the inactive X chromosome as a marker of transcriptionally silent chromatin. We hypothesize that a number of protein factors will fail to localize correctly in some RTT cells due to the mutation in MECP2. We will introduce a normal copy of MECP2 into the RTT cell lines and investigate the effect on protein localization. In addition, we aim to identify a representative sample of transcripts that fail to be silenced in RTT cell lines and to monitor the degree of silencing that occurs when a normal MEPC2 gene is reintroduced.

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Karen Monier, Ph.D.

The Scripps Research Institute

"Role of MeCP2 in the formation of centric heterochromatin compartments in neurons."

Mentor: Kevin F. Sullivan, Ph.D.

2-Year Award: \$90,000

Lay Final Report (November, 2002)

During my two-year fellowship I depicted in great details the nuclear core anatomy of cells devoted to become brain cells. Because of their potential relationship with Rett Syndrome, my focus was on specific regions involved in keeping gene silenced and thus preventing proteins to be produced. I found that the composition of those regions is not everlasting but is rather well modified during the course of the cell cycle or the course of neuronal differentiation. When cells were pushed towards neuronal differentiation they underwent a dynamic nuclear reorganization over few weeks, associated with MeCP2 expression and a nuclear redistribution of the potential binding sites of MeCP2. My results suggest that the flavor of silenced regions is different in brain cells and is finely tuned by the presence of MeCP2 to keep gene silenced. The absence of a functional MeCP2 that cannot bind to methylated DNA or recruit a repressor will change this delicate balance toward permissive gene activation and therefore modify the neuronal pattern of gene expression. This model supports gene silencing defects in Rett Syndrome patients and provides insights into its mechanistic basis.

The defective gene in Rett Syndrome (RTT) has recently been shown to encode MeCP2, a protein involved in silencing the expression of inappropriate genes in differential cell types. One aspect of gene silencing is that it appears to involve the placement of genes into specific compartments in the nucleus of the cell, where proteins required for gene silencing are concentrated. In neurons, evidence points to the idea that silencing compartments are highly structured and exhibit different organization in different neuronal cell types. This project will evaluate the hypothesis that MeCP2 functions in assembling silencing compartments and that the RTT results from failure to properly assemble these compartments. High-resolution 3-dimensional microscopy will be used to study the structure and molecular composition of silencing compartments in brain tissue from mice, normal humans and RTT patients. In addition, the formation of silencing compartments will be studied in live cells undergoing differentiation into neuronal cells, to directly study the role of MeCP2 in compartment formation. Finally, mutated MECP2 genes will be expressed in neuronal cell model systems to examine the impact of RTT mutations on compartment assembly and composition.

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Christopher Rankin, Ph.D.

National Institute of Neurological Disorders and Stroke (NINDS, NIH)

"Embryonic Stem Cells in the Study of Rett Syndrome Neuropathology"

Mentor: Ronald McKay, Ph.D.

2-Year Award: \$80,000

Lay Summary Report:

Rett Syndrome is a neurodevelopmental disorder characterized by progressive cognitive impairment and ataxia. This disease is caused by mutations in the X-linked gene, methyl-CpG-binding protein (MeCP2). The mechanisms leading to neurological dysfunction in Rett Syndrome patients are not well understood.

Pluripotent embryonic stem (ES) cells isolated from the mouse blastula give rise to all differentiated cell types within the mouse and are amenable to genetic manipulations, such as transgene insertion and targeted gene knockouts. In this laboratory, a system has been developed to direct the stepwise differentiation of ES cells first into neural precursors and then into functional neurons and glia. The initial goal of this proposal was to evaluate this system as a model for Rett Syndrome through the use of ES cell lines which carry a mutation in the MeCP2 gene (kindly provided by Dr. Adrian

Bird, University of Edinburgh).

Initial experiments using ES cells that carry a mutation in the MeCP2 gene demonstrate that MeCP2 is not required in the formation of neural progenitors, neurons or glia in our system. In addition, MeCP2 mutant ES cells are able to form synapses and differentiate into dopaminergic and serotonergic neurons. Future experiments will focus on examining other aspects of neuronal differentiation, such as neural morphology and electrophysiological responses.

Lay Summary

Rett Syndrome is caused by mutations in the X-linked gene, methyl-CpG-bindingprotein2 (MECP2) and primarily affects females. The mechanisms leading to neurological dysfunction in Rett Syndrome (RTT) patients are not well understood. Investigation of possible therapies for RTT will require the development of suitable models to study the cellular and neurological consequences of mutations in the MECP2 gene.

Pluripotent embryonic stem (ES) cells isolated from the mouse blastula give rise to all differentiated cell types within the mouse and are amenable to genetic manipulations, such as transgene insertion and targeted knockouts. ES cells can also differentiate in tissue culture to give rise to functional neurons. This experimental system is potentially powerful as the differentiation and function of genetically manipulated cells can be defined both in vitro and in vivo. The in vitro permits a detailed analysis of mechanisms that can be confirmed in vivo.

The aims of the project are:

1. To extend the use of embryonic stem (ES) cells as a source for the major neuronal and glial cells found in the central nervous system.
2. To develop routine assays using ES cell derived neurons to define different steps in neural development and to test the in vivo potential of neurons derived from ES cells.
3. To determine the role of the MECP2 gene in the differentiation, function and survival of neuron and glial cells.

These experiments will determine if the mutation in the MECP2 gene disrupts the basic feature of neural development; including stem cell differentiation and synapse formation. There is neuropathological data suggesting that mutation of the MECP2 gene causes widespread changes in the development of the nervous system. The goal of this research is to define the nature of the developmental defect in RTT.

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Dirk Schubeler, Ph.D.

Fred Hutchinson Cancer Research Center

"Characterization of MeCP2, MBD1 and MBD2 Binding and Activity In Vivo Using Targeted Methylation"

Mentor: Mark Groudine, M.D., Ph.D.

2-Year Award: \$100,000

Lay Final Report (2003):

Rett Syndrome results from mutations in the gene that encodes the protein MeCP2. MeCP2 is thought to play a role in inhibiting the transcription of DNA that is methylated. During the two years of this fellowship we have made significant progress in studying DNA methylation and the role of MeCP2 in methylation-mediated repression. We have shown that MeCP2 binds specifically to methylated DNA in the cell and that this binding coincides with the inhibition of transcription. While these experiments support the current model of how MeCP2 functions, they do not allow the identification of all genes that are bound by MeCP2 and that are potentially mis-regulated in patients with RETT syndrome.

Thus we have developed new approaches to study DNA methylation and protein binding on a genome-wide scale using micro-arrays. Such technology should allow a global but detailed view of the activity of MeCP2 in normal cells and cells from patients with RETT syndrome.

Lay Summary

Mutations in the gene MECP2 result in Rett Syndrome. Normally, the protein MeCP2 binds to methylated DNA and blocks gene activity. Biochemical studies indicate that two additional proteins, MBD1 and MBD2, have similar activities, suggesting that these proteins act together with MeCP2 to regulate methylated genes. To understand how mutations in the MECP2 gene can lead to Rett Syndrome we need to determine the function of MeCP2 and the related proteins MBD1 and MBD2.

Our research focuses on how these proteins bind to methylated DNA in the cell, and how this binding results in the inactivation of the genes. Specifically, we want to determine the extent of methylation required for binding, and to study the molecular steps leading to the repression of gene activity. We have developed a method that allows us to introduce DNA, methylated in the test tube, into specific sites in the genome of living cells. As the methylation state of the DNA is predetermined, we can accurately study the interplay between methylated DNA and the proteins that bind such DNA.

These studies will help us understand the molecular basis for Rett Syndrome and aid in the identification of genes that are regulated by MeCP2.

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