

Research Awardees: 2002

Research Awards

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Research Awards

Schahram Akbarian, M.D., Ph.D.

University of Massachusetts Medical School

"Post-Translational Modifications of Histones in Cerebral Cortex of Normal and of MeCP2-deficient Mice"

2-Year Award: \$85,000

Research Sponsor: The Massachusetts Rett Syndrome Association

Final Report (November 2004)

The Rett disease gene, MECP2, encodes a protein that is thought to regulate chromatin structure and function. Chromatin is comprised of the genomic DNA and a variety of molecules that bind to it. These includes a type of proteins, called the histones, which are involved in the three-dimensional organization of DNA and chromatin structures. A number of different chemical „tags%, or modifications, located at the tails of the histone proteins defines chromatin function. For example, if a gene is expressed at high levels in a particular cell, typically the surrounding histones are acetylated (acetylation is one of several chemical tags).

Current models on MECP2 function predict that this protein regulates histone acetylation in brain. Presently, many studies have been published describing the regulation of histone acetylation in cell culture systems. However, very little

is known about the molecular mechanisms that regulate histone acetylation and other types of modifications, such as phosphorylation, in the brain of a living animal (= in vivo). With the support from RSRF, my laboratory undertook the effort to study how acute changes in dopamine receptor activity (dopamine is a messenger molecule for brain cells) affect histone acetylation and phosphorylation. We were able to show that acute blockade of dopamine (D2) receptors results in a striking short-term increase in levels of histone phospho-acetylation in certain subregions of the brain. Furthermore, we found out that in contrast to these impressive changes in drug-treated animals, levels of histone modifications in mutant mice with a genetic deletion of *Mecp2* in brain after birth were surprisingly normal. This finding may suggest that, under certain conditions, loss of *Mecp2* function does not result in a generalized alterations in chemical histone modifications in the brain.

Lay Summary

Recent years have brought remarkable progress in our efforts to understand the biology of Rett Syndrome, a neurological condition with onset in early childhood. Geneticists have now identified the Rett gene, *MECP2*, and work done by molecular biologists provided some insights into the function of MeCP2 inside a cell. However, we still don't know why loss of MeCP2 function ultimately leads to brain disease. The focus of this proposal is on the 'histones'. These are proteins that, together with the DNA that is wrapped around them, form the fundamental unit of 'chromatin'. The chromatin essentially encodes our genes and controls the correct expression of these genes. MeCP2, in turn, is thought to modify chromatin function but it is still a mystery what exactly the role of MeCP2 is in brain cells. We use a technique (called the 'thalamo-cortical slice preparation') by which the histones in living cells can be experimentally manipulated, and we will use this technique in order to get insight into the molecular mechanisms that regulated histone chemistry and we will search for abnormalities in brain cells of MeCP2-deficient mice. The 'thalamo-cortical slice preparation' may thus provide crucial clues on how MeCP2 mutations may lead to brain disease in Rett Syndrome and may also turn out to be a very useful tool for pharmacologists and other researchers that look for drugs or other ways to cure Rett Syndrome.

Abstract

It is still unclear how deleterious mutations of MeCP2, the Rett gene, result in neuronal dysfunction and brain disease. MeCP2 is associated with chromatin-remodeling complexes that, among other functions, regulate post-translational modifications of several histones. Interestingly, non-neuronal MeCP2-deficient cell lines show hyperacetylation for selected histones. However, it is yet unclear if MeCP2-deficient neurons show abnormalities in acetylation, methylation or other site-specific modifications at the amino-terminal tails of histones, which are the key regulators for chromatin structure and transcriptional activity. Based on our previous work on the neuropathology of MeCP2-deficient mice, the focus of our current proposal is on covalent modifications at the NH₂ terminal tails of histones H3 and H4 in neurons of the cerebral cortex, a brain region known to be affected in Rett. Furthermore, we introduce the thalamo-cortical slice preparation of the mouse as a model system to induce, under controlled conditions in vitro, dynamic changes in the composition of the NH₂ terminal tails of histones H3 and H4. We will then use the thalamo-cortical slice preparation in order to dissect the molecular mechanisms that regulate these dramatic changes in histone composition in normal brain and study potential alterations in the MeCP2-deficient brain. In addition, we will conduct a detailed study on the developmental regulation of site-specific modifications of histones H3 and H4 in the cerebral cortex.

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John Aletta, Ph.D.

University of Buffalo School of Medicine

"Post-Translational Modification of MeCP2 by Arginine Methylation"

2-Year Award: \$95,120

Research Sponsor: Family and Friends of Jessie Lebson

Final Report (November 2004)

The function of the protein product of the MeCP2 gene is likely regulated by chemical modification in living cells. There is a distinct amino acid signature in the MeCP2 protein that is suggestive of modification by the addition of methyl groups to the amino acid arginine. The Aletta laboratory carried out experiments to determine if MeCP2 is indeed modified in intact cells by this means of chemical modification. The results demonstrate that three such methylation sites exist in MeCP2. The methylation occurs in living cells and can be increased by treatment with a class of neuronal growth factors known as neurotrophins. Whereas evidence from the Greenberg lab indicates that chemical modification by phosphorylation decreases MeCP2 binding to DNA, the effect of increased protein methylation of MeCP2 promotes DNA binding. Future experiments will be aimed at the examination of the complementary roles of protein methylation and phosphorylation in regulating the function of MeCP2 in neuronal gene expression.

Lay Summary

Rett Syndrome is a neurodevelopmental disorder that is caused by mutations in the MECP2 gene. Little is known, however, about the precise function of normal MeCP2 protein in neuronal cells. Our preliminary work indicates that the MeCP2 protein is a likely target for enzymes that alter the chemical structure of MeCP2 by protein methylation. During neuronal differentiation induced by nerve growth factors, it has been demonstrated that this class of enzymes is activated. We hypothesize that MeCP2 is an excellent candidate modulator that helps to control the expression of neuron-specific genes. We further hypothesize that the methylation of MeCP2 is a likely chemical "switch" that is thrown to facilitate neuronal development.

This proposal seeks to analyze the regulation of MeCP2 in a well-established model of neuronal differentiation that is easily manipulated under clearly defined cell culture conditions. In this manner, the cellular and molecular consequences of methylating MeCP2 for the regulation of neuronal differentiation can be examined in more detail. We will study whether the methylated form of MeCP2 binds to DNA differently and whether this chemically modified form of the protein affects the expression of essential neuronal genes. The results will provide fundamental information necessary for interpreting the neuronal dysfunction of Rett Syndrome. It is hoped that the new insights into MeCP2 neurobiology acquired from this work will foster novel approaches to the pharmacological treatment of the disorder.

Abstract

This project is based on theoretical and in vitro evidence that indicates the protein product of the MECP2 gene is a methyl protein. The principal hypothesis of this proposal is that the arginine methylation of MeCP2 and its regulation by NGF are important for the normal cellular function of MeCP2 during neuronal differentiation. The specific aims are (1) Demonstrate that MeCP2 is post-translationally modified by arginine methylation in cultured cells and rat brain. We have confirmed that there are several consensus arginine methylation sites in MeCP2 (in human, R162, R186, and R188) by in vitro methylation studies. (2) Determine whether neuronal differentiation affects the methylation status of MeCP2. Previous work in my lab demonstrates that NGF regulates protein arginine methylation. Based on the information in Background and Significance, we expect that the arginine methylation of MeCP2 plays a role in regulating transcription-related events during neuronal differentiation. (3) Determine the effect of MeCP2 methylation on protein function. The effects of altered methylation of MeCP2 on DNA binding and transcription from adenovirus major late promoter and neuron-specific methylated DNA templates will be assessed.

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Rudolf Jaenisch, M.D.

Whitehead Institute for Biomedical Research

"Mecp2 Deficiency in Mice: Can the Course of RTT-like Disease Development be Modified?"

2-Year Award: \$100,000

Lay Progress Report (August, 2003)

We are using the MeCP2 mutant mice as a model system to study the molecular mechanism of human Rett Syndrome and to design therapeutic approaches. We have focused on identifying target genes of MeCP2, and assessing whether any of them is of therapeutic potential. We have found BDNF (Brain Derived Neurotrophic Factor) to be such a gene. We have reason to believe that BDNF might play a role in the progression of RTT disease. We are doing more research to test whether manipulating BDNF expression in MeCP2 mutant mice can slow down disease progression. We are also using various transgenic approaches to reactivate Mecp2 in mutant mice at different stages of disease progression.

Lay Summary

One of the most important and as yet unresolved issues in the pathoetiology of RTT is whether the disease is caused by 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 phenotypic manifestation in the CNS. The main focus of the proposed research is to establish an experimental system that allows the manipulation of the expression of Mecp2 and other interacting proteins in the brains of Mecp2 mutant animals. We expect that the results obtained from these experiments will provide information that is relevant for understanding the pathology of RTT disease development. Of potential therapeutic significance is the question of whether mutant neurons are normal at early postnatal ages and become dysfunctional only later. For example, the possible result that activation of Mecp2 in mutant animals before overt onset of disease ameliorates or even prevents the disease would suggest that no irreversible functional impairment has yet occurred in the neurons of a newborn individual carrying the mutation. This would be an important finding as it would provide a rational strategy for designing therapeutic approaches for RTT with the goal of preventing the disease from developing or ameliorating the severity of the disease phenotype. For example, using pharmacological approaches, such a strategy could consist of altering components other than Mecp2 that affect chromatin conformation with the aim of counteracting the impaired chromatin silencing that is caused by Mecp2 deficiency.

Abstract

We have generated a model for RTT syndrome by deleting Mecp2 in mice. Mecp2-null mice are normal until about 5 weeks of age when they begin to exhibit abnormal behavior that bears some resemblance to symptoms observed in girls with RTT syndrome. The mutants usually die between 6 and 8 weeks of age. Using the mutant mice as a model system, the focus of our work is to understand the molecular and biological consequences of Mecp2 deficiency. We propose the following projects: 1) We will combine DNA hypomethylation of Bdnf deficiency with Mecp2 deficiency to identify possible interacting factors. 2) We will compare mutant and control neurons in an in vitro culture system in an effort to develop functional markers that distinguish mutant from control neurons. 3) We will construct mice carrying inducible Mecp2 transgenes that will allow the activation of the protein in neurons of mutant mice at different times prior to onset of overt disease. Conversely, we will derive mice that permit the inactivation of Mecp2 at different postnatal ages.

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Hannah Kinney, M.D.

Harvard Medical School

"Serotonin in the Rett Medulla"

1-Year Award: \$50,000

Research Sponsor: Family and Friends of Lauren Stevens

Final Report (November 2004)

In this study we demonstrate that the level of serotonin transporters in the dorsal motor nucleus of the vagus and the raphé obscurus in the medulla oblongata decreases significantly with age in control cases but not in Rett patients. These regions in the medulla are critical for the unconscious control of normal breathing, heart rate, blood pressure and temperature and are regulated by serotonin, a naturally occurring brain chemical. The serotonin transporter, which is present only on neurons containing serotonin, controls the amount of serotonin available in the brain by the uptake (transport) of free serotonin back into the neuron. A change in the levels of serotonin transporter in the brain is therefore an indication of abnormal levels of serotonin or serotonin neurons. In this regard, we counted the number of serotonin neurons in the medulla oblongata of Rett and control patients, but observed no significant difference. These observations indicate that while there is no change in the number of serotonin neurons, subtle abnormalities in the development of the serotonin system are present in Rett patients.

This study was performed using tissue autoradiography on frozen medullae of 6 Rett and 6 control cases. While still frozen, the medulla from each case was cut into thin slices and collected onto glass microscope slides. The slides were then incubated in a solution containing 125I-RTI-55 a drug that binds to serotonin transporters that is labeled with radioactive iodine (125I). The sections were then removed from the solution rinsed, dried and then placed against photosensitive film for a pre-determined time. The radiation emitted by the 125I label attached to the RTI-55 reacts with the film producing an image of the medulla upon development. Using computer aided quantitation the density of the serotonin transporter binding in individual regions of the medulla in each Rett and control case was then measured from the film. The transporter binding levels for each region were averaged for all Rett and all controls cases and statistically analyzed to determine if binding levels were different in Rett cases compared to controls. Results of the analysis revealed that serotonin transporter binding decreased significantly with age in the medulla oblongata in control cases but not in Rett patients.

Rett syndrome is a disorder of brain development characterized by gradual cognitive decline following normal development through the first 6-18 postnatal months and is one of the most common causes of mental retardation in females. In addition, Rett patients demonstrate breathing difficulties, abnormal heart rate variability and sudden death. The observations in this study suggest that abnormal levels of serotonin transporter may be responsible for the problems with breathing and heart rate, and incidence of sudden death, associated with Rett. In addition, serotonin plays an important role in the normal development of the brain. Changes in serotonin levels may therefore also be responsible, at least in part, for abnormal brain development in Rett.

Lay Summary

The Rett child and adult frequently exhibit irregular patterns of breathing which appear to cause discomfort and which may, in some cases, be dangerous. The control of breathing requires a complex interaction of neurons in the brainstem. This interaction is mediated by chemicals that are produced in specific neurons in the brain. Serotonin is one of these chemicals. Its action by appropriate neurons requires enzymes, transporters and receptors in appropriate groups of neurons within the respiratory control circuit. The chemical mediators in the brain, such as serotonin, have potent and specific effects on brain function. Their effectiveness depends upon being produced in appropriate neurons at the correct times. Regulatory proteins, such as MeCP2, ultimately control the expression of the chemical mediators in specific cells. It is known that MeCP2 is deficient in some neurons in the Rett brain. In this study we hypothesize that there is an abnormality of serotonin synthesis, transport and/or receptors in neurons involved in respiratory control, and that these neurons are also deficient in MeCP2. If a specific deficiency in the serotonin is identified, specific drugs to normalize breathing could be designed, thereby improving the quality of life of patients and potentially reducing their risk

for sudden death.

Abstract

We hypothesize that MeCP2 deficiency influences serotonin's role in the control of respiration causing breathing irregularities in Rett Syndrome (RTT). Using autoradiography applied to frozen brainstems from RTT and control autopsies, we will determine the level of expression of serotonin receptors and transporters in the caudal raphe nucleus and its major projection sites to brainstem nuclei involved in respiratory control. Using immunocytochemistry the same respiratory-related nuclei will be examined for tryptophan hydroxylase (the enzyme required for serotonin synthesis) and for MeCP2, the regulator protein that is deficient in RTT. The identification of specific abnormalities in serotonin synthesis, uptake and/or receptor binding may help determine strategies for correction of the breathing irregularities in RTT.

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Janine LaSalle, Ph.D.

University of California at Davis

"The Role of MeCP2 in the Ontogeny of Cerebral Cortical Neurons"

1-Year Award: \$50,000

Research Sponsor: Reading Rock Inc.

Lay Progress Report (August, 2004)

Rett syndrome is caused by mutations in the gene MECP2 that encodes a protein, methyl-CpG protein 2 (MeCP2). Elevated MeCP2 expression is acquired in individual neurons within the brain beginning in infancy and progressing throughout childhood. The function of MeCP2 in the developing brain is unclear at this stage, but the mutations in Rett syndrome and the Mecp2 "knockout" mouse model provide evidence that MeCP2 is essential for mature neuronal function. MeCP2 is predicted to be a regulator of other genes in maturing neurons, but finding these genes is complicated by the complexity of cells and genes in the brain. The first aim of this proposal was to use a cell culture system for inducing neuronal maturation to identify genes expressed in a single cell type at a precise time. MeCP2 activity was blocked by using a "decoy" inserted into the cells. Genes that show significantly altered expression levels in cells with blocked MeCP2 activity were identified by "gene chip" technology. In the second aim of this proposal, candidate genes identified in Aim 1 will be tested for expression patterns in normal and MECP2/Mecp2 mutant human and mouse brain samples. An automated approach of quantitating proteins in multiple tissue samples by laser scanning cytometry has recently been developed by the PI and will be used to test the effect of MECP2/Mecp2 mutations on the normal developmental expression of the candidate genes. The results from these studies are expected to provide new information for understanding how MECP2 mutations cause Rett syndrome and provide multiple novel molecules that could be targeted for therapeutic intervention.

Lay Summary

Rett Syndrome is caused by mutations in the gene MECP2 that encodes a protein, methyl-CpG protein 2 (MeCP2). Elevated MeCP2 expression is acquired in individual neurons within the brain beginning in infancy and progressing throughout childhood. The function of MeCP2 in the developing brain is unclear at this stage, but the mutations in Rett Syndrome and the Mecp2 "knockout" mouse model provide evidence that MeCP2 is essential for mature neuronal function. MeCP2 is predicted to be a regulator of other genes in maturing neurons, but finding these genes is

complicated by the complexity of cells and genes in the brain. The first aim of this proposal is to use a cell culture system for inducing neuronal maturation to identify genes expressed in a single cell type at a precise time. MeCP2 activity will be blocked by using a "decoy" inserted into the cells. Genes that show significantly altered expression levels in cells with blocked MeCP2 activity will be identified by "gene chip" technology. In the second aim of this proposal, candidate genes identified in Aim 1 will be tested for expression patterns in normal and MECP2/Mecp2 mutant human and mouse brain samples. An automated approach of quantitating proteins in multiple tissue samples by laser scanning cytometry has recently been developed by the PI and will be used to test the effect of MECP2/Mecp2 mutations on the normal developmental expression of the candidate genes. The results from these studies are expected to provide multiple novel molecules that could be targeted for therapeutic intervention.

Abstract

Rett Syndrome is an X-linked dominant neurodevelopmental disorder caused by mutations in MECP2, encoding methyl-CpG protein 2 (MeCP2). MeCP2 selectively binds to methylated CpG residues and is hypothesized to be essential during neuronal maturation in the postnatal central nervous system. The first aim of this proposal is to use gene expression microarray analysis to identify novel gene targets of MeCP2 during neuronal maturation. Neuroblastoma cells induced to undergo maturational differentiation will be transfected with MeCP2 decoy to block MeCP2 binding to endogenous targets. In the second aim of this proposal, genes with significantly altered expression levels in cells with blocked MeCP2 activity will be then tested for quantitative protein expression by immunofluorescence and laser scanning cytometry on developmental tissue arrays of normal and MECP2/Mecp2 mutant human and mouse cerebral cortex samples. MeCP2 expression will be compared to each new marker to determine the relationship of the markers in normal neuronal ontogeny and the effect of MECP2/Mecp2 mutation on their normal expression. The results of these studies are expected to be important in determining the causative role of MECP2 mutations in the pathogenesis of Rett Syndrome.

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Judith Neubauer, Ph.D.

University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

"Cardiorespiratory Control in Rett Syndrome"

2-Year Award: \$99,000

Research Sponsor: Schmidlapp Fund

Lay Progress Report (August, 2003)

RTT is associated with significant respiratory disturbances indicating that there is some inability of the respiratory centers in the brain "to stabilize" the breathing pattern. Breathing is usually a fairly rhythmic process and doesn't exhibit periods of rapid breathing followed by periods of reduced breathing as can occur in RTT. How the respiratory center stabilizes breathing normally and why it doesn't in RTT, is the focus of our research project funded by the RSRF. Our previous work has suggested that the stability of breathing may require the ability of the respiratory center to produce large breaths (sighs), which act to "reset" the pattern should it be deviating from the rhythmic pattern. Everyone generally sighs about six times an hour and this presumably is sufficient to keep the pattern at the desired set point. In addition, the number of sighs increases under conditions like hypoxia, since hypoxia may increase the tendency of the pattern to either overshoot or undershoot a set point. In order to determine if the impaired ability to stabilize breathing in RTT is linked with an inability to increase sighs, we have taken advantage of the Mecp2 knockout mouse to study the stability of breathing during conditions that would normally increase sighs. Our initial results have been very exciting. We have found that the Mecp2 knockout mouse does not have spontaneous sighs nor does it sigh even when exposed to hypoxia. One is always cautious when interpreting preliminary results, but if this observation can be confirmed with

repeated experiments it may suggest potential strategies for treating respiratory disturbances in RTT. For example, we now know the part of the brain that is necessary to produce sighs, a part of the respiratory center called the pre-B^ztzinger Complex. What is of particular interest, is that another RSRF funded investigator (Dr Ramirez) has shown that the pre-B^ztzinger Complex in Mecp2 knockout mice is lacking the appropriate number of receptors for a substance called substance P, which may be essential for activation of this site to generate sighs. Taken together, this may lead to identification of drugs that could selective increase the ability of the pre-B^ztzinger Complex to produce sighs and, thereby, stabilize the breathing pattern.

Lay Summary

Rett Syndrome is a neurodevelopmental disorder that occurs in 1 out of every 10,000 to 15,000 girls. Girls afflicted with Rett Syndrome can develop severe mental retardation with impaired motor skills within the first two years of life. In addition to these symptoms, girls with Rett have profound breathing and heart rate irregularities while awake that become exaggerated when they become excited or anxious. In 1999, a genetic mutation for Rett Syndrome was identified. This gene is important for producing a protein (MECP2) which regulates the production of other factors that are presumably necessary for normal brain function. However, the exact mechanisms for how MECP2 regulates normal brain development are unknown. The identification of the gene has now resulted in the development of a mouse that lacks this gene, the Mecp2 knockout mouse. This mouse can be used to test whether the loss of this gene causes the same disturbances in function that are seen in girls with Rett Syndrome. The studies in this proposal will examine these Mecp2 knockout mice to determine whether they have the same breathing and heart rate problems seen in girls with Rett. In addition, the studies will test novel responses of these mice to conditions of lowoxygen and high carbon dioxide and use this information to predict how girls with Rett Syndrome may respond to clinical conditions that might result in low oxygen or high carbon dioxide, such as occur during respiratory or cardiovascular diseases. Ultimately, these studies will provide important information that could be used to develop new therapies for patients afflicted with Rett Syndrome.

Abstract

Rett Syndrome (RTT) is characterized by a number of neurodevelopmental abnormalities including dysfunctions of the autonomic nervous system with irregularities of respiration and heart rate. Recently, mutations in the gene encoding MeCP2 have been identified in patients with RTT leading to the development of a genetically engineered mouse model, the Mecp2 knockout mouse. The Mecp2 null mouse offers a unique opportunity to determine whether this mouse model mimics the clinical phenotypes of RTT as well as an ability to extend the current state of knowledge regarding the neurophysiological processes. Thus, the major goals of the studies proposed in this application are two-fold: (1) to determine whether Mecp2 null mice exhibit respiratory irregularities and instability as well as diminished evidence of autonomic coupling in heart rate variability under baseline conditions; and (2) determine whether these irregularities are enhanced when the Mecp2 null mice are challenged with hypoxia and hypercapnia and further whether these mice fail to adapt to chronic hypoxia. Mecp2 null mice will be chronically prepared with diaphragm EMG and EKG electrodes to monitor respiration and heart rate variability. The results of these studies will define the cardiorespiratory characteristics of the Mecp2 knockout mouse and clarify the link between the MECP2 gene and the clinical phenotype of RTT.

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Weidong Wang, Ph.D.

National Institute of Aging, NIH

"Characterization of the MeCP2 Complex Involved in Rett Syndrome"

2-Year Award: \$100,000

Research Sponsor: Family and Friends of Gabriela Southren

Final Report (November 2004)

The Majority of Rett syndrome patients are defective in the MeCP2 gene. This gene encodes a protein that plays a key role in regulating gene expression. Elucidating the mechanism of how MeCP2 controls gene expression is highly critical for understanding of the Rett syndrome. The objective of our proposal is to study this mechanism.

It is known that many proteins in cells do not function by themselves. Instead, they associate with other proteins to work together like a concerted machine. The overall function of the machine is determined not by one protein, but by all the protein components. Thus, to fully understand function of a given protein, one must identify and investigate the machines which the investigated protein is part of. Because the cell usually expresses thousands of genes, there will be thousands of proteins and protein machines present in cells. Technically, it is quite a challenge to isolate a specific machine away from thousands of others. Our lab has recently developed a highly efficient method to isolate such machines from cells. For example, we have isolated and characterized protein machines involved in a different mental retardation disease, ATRX-syndrome, as well as those involved in Bloom syndrome, Rothmund-Thomson syndrome and Fanconi anemia.

Previous studies have suggested that MeCP2 could be part of a multi-protein machine that regulates gene expression through a specific enzyme (called histone deacetylase). But this machine has not been isolated by unbiased biochemical approaches to a degree of purity that would allow us to understand its structure or function. We have applied our established method and isolated MeCP2 from human cell extract and mouse brain extract under very mild conditions, with the hope to retain the potential MeCP2 machines as intact as possible. The purity of our MeCP2 preparation is very high, and we were able to unequivocally identify MeCP2. To our surprise, we found that MeCP2 does not appear to form a stable machine with any other proteins. In particular, we found no evidence that MeCP2 associates stably with the previously described enzyme. Another report this year from Adrian Bird's paper reached a similar conclusion. Based on these studies, we suggest that MeCP2 probably does not directly work through this enzyme as many people have believed.

Interestingly, we found that our biochemically isolated MeCP2 is modified by a mechanism called phosphorylation. It has been well established that phosphorylation is a common mechanism for cells to reversibly regulating activity of a protein. A report from Mike Greenberg's lab has shown that phosphorylation of MeCP2 plays a critical role in regulating its activity in neurons. We were able to identify a single phosphorylation site on MeCP2 protein isolated from several different sources, including human cells, mouse and rat brain. We show that the level of this phosphorylation is regulated when cells are at different stages of growth: it is very low when cells synthesize their DNA, and becomes very high when cells divide. Our study suggest that phosphorylation of MeCP2 at this particular site is important to regulate its function during cell growth.

Lay Summary

The majority of Rett Syndrome patients are defective in the MECP2 gene. This gene encodes a protein that plays a key role in regulating gene expression. Thus, the cause Rett is most likely due to inappropriate expression of the genes that are under the control of the MeCP2 protein. These genes may normally be turned off in healthy individuals. But in Rett patients, the genes become turned on because of defective MeCP2 protein. If any of these genes are required for normal development of brain or other nerve organs, their inappropriate expression may lead to developmental abnormalities and mental retardation. Elucidating the mechanism of how MeCP2 controls gene expression is therefore highly critical for understanding Rett Syndrome. The objective of our proposal is to study this mechanism. It is known that many proteins in cells do not function by themselves. Instead, they associate with other proteins to work together like a concerted machine. The overall function of the machine is determined not by one protein, but by all the protein compartments. Often, one protein could participate in several different machines, with each machine having its own function. This way, one protein could perform multiple jobs for the cell. Thus, to fully understand function of a given protein, one must identify and investigate the machines which the investigated protein is part of. Because the cell usually expresses thousands of genes, there will be thousands of proteins and protein machines present in cells. Technically, it is quite a challenge to isolate a specific machine away from thousands of others. Our lab has recently developed a highly efficient method to isolate such machines from cells.

Previous studies have suggested that MeCP2 could be part of a multi-protein machine. But it has not been isolated for us to completely understand its structure or function. In this proposal, we plan to use our established method to completely purify this machine, identify all its components (which could include genes that themselves cause Rett Syndrome) and analyze its function.

Abstract

With the demonstration that up to 80% of cases are caused by mutations in a methyl-DNA-binding protein, MeCP2, the analysis of neurological effects and mental retardation in Rett Syndrome must focus on associated changes in chromatin. The working hypothesis is that chromatin remodeling based on methylation must be modified to turn on or off critical proteins active in brain function. Almost certainly, a multiprotein complex containing MeCP2 is involved, for it is becoming increasingly clear that chromatin remodeling, like many reactions in the cell nucleus, is carried out by such complexes. Often, one protein can be part of several complexes, with each complex having a unique function. In fact, MeCP2 has been shown to co-fractionate and coimmunoprecipitate with histone deacetylase HDAC1 and transcription corepressor mSin3A, proteins that are presumably part of the complex. However, the entire complex (or complexes) containing MeCP2 has not been completely purified or characterized. We want to apply purification procedures that we have developed and successfully used to analyze other disease-related chromatin remodeling complexes to identify the protein partners of MeCP2 in the cell. The proximal goal of this research is to understand the function of MeCP2 in vivo; the long-term goal is to be able to intervene to substitute for MeCP2 or compensate its loss.

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Debra Weese-Mayer, M.D.

Rush Children's Hospital at Rush-Presbyterian-St Luke's Medical Center

"Breathing Disorder in Rett Syndrome and the Malregulation of Autonomic Function"

2-Year Award: \$100,000

Research Sponsor: Gardner Family Foundation

Lay Progress Report (August, 2003)

With the generous allocation of research funding the proposed research project is continuing to evolve and grow. Many changes have been initiated since the project inception. First, we are expanding the age range from ages 2-5 years, now to 2-6 years. Second, we are transitioning to a totally inclusive vest technology to simplify the task of recording. This is a remarkable improvement over the 13+ pound monitor on the luggage cart! We are in the final stages of verifying that the ASCII file output from the LifeShirt vest can be fully interpreted for non-linear analysis. As soon as that is completed we will be ready to zip the girls into the vests and simplify the recording experience for their families. Third, we have modified the duration of time for monitoring to include 2 hours awake, for 2 consecutive days, watching a specific video that we will include as a gift with the equipment shipment. We are still requesting two complete nights of recording. This revision in the protocol now has the Rush Institutional Review Board approval. This modification will be more realistic for parents who are already juggling too many responsibilities and who are graciously participating in our research project.

Please remember that for enrollment in our study, we need a copy of the laboratory report indicating the genetic testing results for one of the known Rett mutations. After a family signs the IRB approved consent and the HIPAA consent, we

will send them a request for medical records. We will also arrange for a mutually agreeable time to use the recording equipment. When the equipment is shipped we will include clear understandable directions for its use, an activity log to complete during monitoring the child, and the educational video. In addition to assistance from our new Project Coordinator, Ms. Christina Boothby, there is a 24 hour help line for the LifeShirt vest technology company. Parents should never feel alone when they are participating in our research study!

We are continuing to look at the waveforms and scoring by the traditional method, and by the non-linear analysis. As the sample size grows we anticipate preparing a preliminary report for RSRF. At this point, the data sample is too small to confidently report our findings.

Lay Summary

Our primary objective is to determine the relationship between breathing, heart rate, and oxygenation in girls with Rett Syndrome. These all represent systems that function automatically in the healthy child, but that appear to represent malregulation in the child with Rett Syndrome. The literature suggests that children with Rett Syndrome have symptoms during wakefulness only. We believe that if the recorded data are analyzed with specific attention to those systems that function automatically, reflecting function of the Autonomic Nervous System (ANS), it will become apparent that children with Rett Syndrome have remarkable symptoms awake and asleep. By studying waveforms recorded non-invasively in each child's own home over 48 continuous hours, then analyzing the waveforms by traditional scoring methods and by a special kind of analysis that allows us to determine the "health" of the ANS, we anticipate finding that these special children experience symptoms of ANS malregulation. Once identified, we will be able to better understand the function of the ANS and propose intervention strategies specific to children with Rett Syndrome.

Abstract

We hypothesize that children with Rett Syndrome will have uncoupling between breathing and measures of heart rate control, oxygenation, and pulse waveform amplitude, indicating malregulation of the Autonomic Nervous System (ANS), and that these findings will be apparent during sleep as well as wakefulness. 50 girls with Rett Syndrome between the ages of 2 ; 5 years of age and 50 control subjects matched for race, sex, age, and body mass index will be included in this study. Each family will receive an instructional videotape teaching the use of the SomnoStar PT2 multichannel home monitor, designed to continuously record inductance plethysmography of the chest and abdomen, ECG, hemoglobin saturation and pulse waveform. Each family will be instructed to use the home monitor on the study child for 48 continuous hours. The waveforms in the home monitor memory will be stored on the designated computer in Dr. Weese-Mayer's laboratory for traditional waveform analysis and an identical copy of the waveforms will be provided to Dr. Ramirez at the University of Chicago for analysis with computer algorithms to detect ANS uncoupling. Statistical analysis of the data scored by traditional analysis techniques will include comparison for the incidence of central, obstructive, and mixed apnea, hypopneas, bradycardia, hemoglobin desaturation, and pulse waveform amplitude decrements (Student's T-test). Statistical analysis of the data scored by ANS uncoupling analysis will initially be descriptive, then involve linear regression models. The results of our proposed study will provide a more clear description of the phenotype of the control of breathing deficit in Rett Syndrome as it pertains to the ANS. If our hypothesis is correct, Rett Syndrome will provide a logical segue to a growing number of diseases that reflect ANS dysfunction (Sudden Infant Death Syndrome, Idiopathic Congenital Central Hypoventilation Syndrome, Familial Dysautonomia). Taken together, the results generated by our proposed study will facilitate/expedite study into the control of the ANS and potential intervention strategies for children with Rett Syndrome.

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Post-Doctoral Fellowship Awards

Deborah Bourc'his, Ph.D.

Columbia University,

Mentor: Timothy Bestor, Ph.D.

"Involvement of DNA Methylation in the Etiology of Rett Syndrome in a Mouse Model"

2-Year Award: \$100,000

Final Report (November 2004)

Rett syndrome is caused by mutations in MECP2, which is considered to be a repressor of gene expression in brain. MeCP2 has a potential function as a mediator of DNA methylation in the genome, by binding to methyl-residues added to genes and converting this signal into silencing of these tagged genes. On this assumption, Rett syndrome has been classified as a methylation-related disease. However, the lack of reactivation of methylated promoters in Rett patients and Mecp2 mutant mice renders questionable the predicted involvement of MeCP2 in methylation-dependent repression. Our goal is to determine whether MeCP2 function really relies on methylation.

The analysis of single versus double-mutants is a classical genetic way to assess a functional link between two factors. To assess the functional interaction between MeCP2 and DNA methylation, we have crossed the Mecp2 mutation onto different methylation-deficient backgrounds in mice, Dnmt1 and Dnmt3L mutant mice. We did not see a detrimental cumulative effect of the two mutations, ruling out an involvement of Mecp2 in global methylation-dependent repression pathway during embryonic development. By applying a large-scale analysis of methylation patterns in brain samples from Mecp2-mutant mice and Rett patients, we also excluded a role of MeCP2 in stabilizing DNA methylation patterns.

Lay Summary

Rett Syndrome is caused by mutations in MECP2, which is considered to be a methylation-dependent transcriptional repressor. MeCP2 has a potential function as a mediator of DNA methylation in the genome, by binding to methyl-residues added to genes and converting this signal into silencing of these tagged genes. On this assumption, Rett Syndrome has been classified as a methylation-related disease, and therefore as an epigenetic rather than a classical neurodevelopmental disease. But while MeCP2 is certainly involved in silencing of specific genes at critical times of brain development, its dependence on methylation is more and more questionable. The most intriguing fact is that repression of methylated genes still operates in mice lacking MeCP2, a mouse model that reproduces remarkably Rett-like symptoms. Moreover, there is no clinical overlap between Rett patients and patients with ICF syndrome, a human genetic disease which has been linked to a methylation defect. Our goal is to determine whether MeCP2 function really relies on methylation. The analysis of mice mutant for both MeCP2 and methylation will enable us to see if MeCP2 mutation has an enhancing effect on methylation deficiency or in other words if MeCP2 is involved in the methylation-dependent repression pathway. This study should answer the question as to whether Rett Syndrome is related to methylation. This is a critical issue to address if we are to elucidate the mechanisms by which Rett Syndrome develops, and to orientate research to find specific therapeutic approaches.

Abstract

Rett Syndrome has been classified as an epigenetic disease based on the fact that the gene responsible for the Syndrome, MECP2, has the in vitro ability to induce repression of methylated promoters. However, there is no derepression of methylated genes in Mecp2 mutant mice, which remarkably reproduce Rett-like symptoms. Our broad objective is to determine whether Mecp2 can still be regarded as a methylation-dependent transcriptional repressor. Crossing the Mecp2 mutation onto different methylation-deficient backgrounds in mice will enable us to genetically assess the functional interaction between methylation and Mecp2. By using three different methyltransferase mutant background, we will investigate the importance of Mecp2 in the methylation system for 1) global methylation-dependent silencing, 2) CNS function, in accordance with the brain specificity of Rett Syndrome and 3) the control of imprinting. This project will address the issue of whether Rett Syndrome is a methylation or a classical neurodevelopmental disorder. This is a crucial precondition to understanding the nature of the neuronal deficits involved in the pathology and

orientating research towards specific therapeutic approaches.

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Peng Jin, Ph.D.

Emory University School of Medicine

Mentor: Stephen Warren, Ph.D.

"Identification of Target Genes of MeCP2 in Neurons"

2-Year Award: \$100,000

Final Report (November 2004)

Rett Syndrome is a neurodevelopmental disorder mainly caused by mutations in the X-linked gene MECP2 and primarily affects females. MeCP2 binds to methylated DNA and blocks gene expression. Mutations in MeCP2 affect its ability to block gene expression and may lead to aberrant patterns of gene expression in RTT. The predominant manifestation of central nervous system dysfunction in RTT suggests that MeCP2 plays a critical role in the development and stability of neurons. However, the downstream target genes regulated by MeCP2 in neurons have not been identified, which will be important for the development of therapeutic approaches. In this study we proposed to use RTT mouse model to identify these target genes. Using RTT mouse model and advanced cell sorting technology, we have isolated specific neurons, Purkinje cells, to study the change of gene expression pattern in the absence of MeCP2 using DNA microarray analysis. Our results suggest that a group of genes involved in protein synthesis were altered in the absence of MeCP2 at day seven, a critical stage for brain development. This indicates that defects in protein synthesis may associate with Rett Syndrome and be responsible for its neurological phenotype.

Lay Summary

Rett Syndrome is a neurodevelopmental disorder mainly caused by mutations in the X-linked gene methyl-CpG-binding protein2 (MECP2) and primarily affects females. MeCP2 binds to methylated DNA and blocks gene expression. Mutations in MeCP2 affect its ability to block gene expression and may lead to aberrant patterns of gene expression in RTT. The predominant manifestation of central nervous system dysfunction in RTT suggests that MeCP2 plays a critical role in the development and stability of neurons. However, the downstream target genes regulated by MeCP2 in neuron have not been identified, which will be important for the development of therapeutic approaches. In this study we propose to use RTT mouse model to identify these target genes. Using RTT mouse model and advanced cell sorting technology, we will isolate the specific neurons, Purkinje cells, to study the change of gene expression pattern in the absence of Mecp2 using DNA microarray analysis. The expressions of more than 30,000 genes will be simultaneously studied and compared between neurons with and without Mecp2. We will also carry out chromatin immunoprecipitation study to identify the genomic region bound by MeCP2 under the physiological condition. By integrating the results from these two different approaches, we will identify the target genes of Mecp2 in neurons. This study will not only provide insight into the molecular pathogenesis of Rett Syndrome but also facilitate the development of effective interventions.

Abstract

Rett Syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in the X-linked gene MECP2, encoding a nuclear protein that selectively binds methylated CpG dinucleotides and mediates transcriptional repression. Both the predominant manifestation of central nervous system dysfunction in Rett Syndrome and neurological phenotypes displayed in the Mecp2-knockout mouse suggest that as a transcription repressor MeCP2 plays critical roles

in the development and stability of neurons. However, the downstream target genes of MeCP2 have not been identified. In this study we propose to use Purkinje cells as model neuron to identify the target genes of MeCP2 in neurons combining both DNA microarray and chromatin immunoprecipitation approaches. The target genes of MeCP2, that display altered expression levels in the absence of MeCP2 and show associations between their own promoter regions and MeCP2 in vivo, will be identified. Identification of MeCP2 target genes will not only provide insight into the molecular pathogenesis of Rett Syndrome but also facilitate the development of effective interventions for Rett Syndrome.

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Juan I. Young, Ph.D.

Mentor: Huda Zoghbi, M.D.

"Is Rett Syndrome Reversible? Conditional Restoration of MeCP2 Function in a Mouse Model for Rett Syndrome"

2-Year Award: \$100,000

Recipient of the Alan P. Wolffe Memorial Fellowship

Lay Progress Report (August, 2004)

MeCP2 mutant mice display a neurological phenotype. To test for the reversibility of this phenotype, we have generated transgenic mice in which MeCP2 could be turned ON and OFF specifically on certain regions of the brain on demand. Mice mutant for the endogenous *Mecp2* gene, that carry the exogenous MeCP2 transgene in its ON version, displayed some improvement, indicating that the transgene is functional. However, the lack of a complete prevention of the phenotype suggests that modifications of transgene expression patterns are necessary. We are generating new mice that will express the transgenic MeCP2 in every neuron in which the endogenous gene is supposed to be present in order to test for a full rescue of the phenotype.

Lay Summary

Animal models of genetic disorders are invaluable for not only understanding disease mechanisms but for testing therapeutic approaches. The discovery of MECP2 as the gene mutated in Rett Syndrome (RTT), and the subsequent generation of a RTT mouse model, have provided the tools to investigate therapeutic strategies that could be applied to treat this severely disabling disease. Several unique features of RTT provide hope that an effective therapy could be developed: the initial period of normal development, the absence of observable neurodegeneration, and the fact that MeCP2 seems to function primarily in mature neurons. We will test the idea that the initial period of normal development displayed by RTT patients indicates that neuronal impairment has not yet occurred, or at least not to an irreversible degree. Thus, we propose to turn on the expression of a functional MeCP2 protein in our RTT mouse model at different stages of disease progression. This will allow us to determine whether any or all aspects of the RTT phenotype can be prevented, reversed, or mitigated.

Abstract

Most children with Rett Syndrome (RTT) undergo a period of apparently normal development. This seems to be due to the fact that MeCP2 is expressed primarily in mature neurons. These two facts provide reason to hope that restoration of MeCP2 function at an appropriate time might prevent neuronal dysfunction and the eventual development of Rett Syndrome. To test this hypothesis I generated transgenic mice that bear a wild-type MECP2 allele whose expression can be induced in the brain at desired time-points. I propose to evaluate the effects of expressing this allele at different stages of disease progression in a mouse model of Rett Syndrome that we have generated. The conditional nature of

the wild-type MECP2 allele will allow us to examine the reversibility of the disease as well as determine the length of time during which therapy is feasible. In addition, I propose to search for genes dysregulated by the loss of normal MeCP2 function that may account for the various aspects of the disease. In summary, I hope to lay a foundation for research aimed at the development of an effective therapy for Rett Syndrome.

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