

Research Awardees: 2004

Research Awards

John Christodoulou, Ph.D.

Charlotte Kilstrup-Nielsen, Ph.D.

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Janine LaSalle, Ph.D.

Sanford Leuba, Ph.D. & Jordanka Zlatanova, Ph.D.

Berge Minassian, M.D.

Sam Pfaff, Ph.D.

Fyodor Urnov, Ph.D. & Egor Prokhorchouk, Ph.D.

Post-Doctoral Fellowship Awards

Skirmantas Kriaucionis

Mizue Hisano, Ph.D.

Satoko Matsumura, D.D.S., Ph.D.

Jean-Christophe Roux, Ph.D.

Matthew Tudor, Ph.D.

Juan Young, Ph.D.

Research Awards

John Christodoulou, Ph.D.

Children's Hospital at Westmead, Sydney

STK9, a second RTT gene: genetic and functional studies

\$50,000

Lay Progress Report (August 2005)

In collaboration with the group led by Professor Angus Clarke and Dr. Hayley Archer, screening of the CDLK5 gene continues in a number of patient groups including those with classical and atypical Rett Syndrome, individuals with intellectual disability, autism and seizures, and families with X-linked mental retardation where seizures (including infantile spasms) figure prominently. A number of sequence variations have been identified, whose pathological significance is currently being evaluated in more detail.

In addition, we are currently examining the sub-cellular localisation of the normal CDKL5 protein in a number of cell types. Once completed, we will then go on to examine the various mutations that have been identified to date, including whether the mutation leads to mis-localisation of the mutant CDKL5 protein, and how the mutation affects protein function.

These studies will yield further studies into the range of clinical presentations associated with CDKL5 mutations, and will help us to understand how these mutations exert their effects at the cellular level.

Lay Summary

Rett Syndrome (RTT) is a devastating progressive disorder affecting motor and intellectual development. It is characterised by normal development for the first 6-12 months of life, followed by developmental regression with the loss of learned purposeful hand function, loss of acquired speech and communicative abilities, sometimes leading to the incorrect diagnosis of autism. It may be the most common cause of progressive mental retardation in girls, with an estimated prevalence in Australia of 1 per 10,000 females under the age of twelve years. It is a genetic disorder and occurs almost exclusively in females.

In 1999, a gene (called MECP2) was identified which appears to be the cause of RTT in most girls and women with RTT. However, for 5 Æ 15% of RTT subjects, no gene change is found in the MECP2 gene, raising the possibility that other genes may also be responsible for RTT.

Our research group has identified one of these genes. Known as STK9, little is known about this gene's function. Of great interest is the fact that our studies suggest that STK9 could also be a cause of intellectual disability in other patients, and with autism.

The focus of this research project is to explore how common gene changes in STK9 are in a large number of children with RTT and inherited intellectual disability. Using cutting edge research technology, we will go on to study how the STK9 protein interacts with MeCP2 and other proteins, in order to better understand how these genes may be detrimentally affecting brain function in girls and women with RTT and other neurological disorders. These studies will give us a greater understanding of normal brain development and function.

Abstract

We have discovered that two MECP2 mutation-negative RTT families have different disease-causing mutations in the gene STK9. Little is known about this putative serine-threonine kinase, but recent studies demonstrating that MeCP2 function is in part a consequence of dynamic changes in phosphorylation, raises the interesting question of whether consequences of STK9 mutations are through direct interactions with MeCP2 or with common pathways that play a role in the maintenance of neuronal plasticity.

Aim 1: To screen patients with neurodevelopmental disorders for mutations in the new RTT gene.

We plan to screen the following cohorts of patients for mutations in this new RTT gene:

a) MECP2 mutation-negative RTT patients from Australia and Cardiff: b) patients with X-linked mental retardation from Australia and Cardiff

Aim 2: To examine the interaction of STK9 with MECP2 and other protein targets.

We are currently in the process of developing antibodies and STK9 expression vectors that will allow us to a) examine the subcellular localisation of wild-type and mutant STK9; b) study whether STK9 interacts directly with MeCP2, and if so at which MeCP2 residues, and whether STK9 modulates the ability of MeCP2 to bind its protein partners; c) determine the proteins with which STK9 interacts upstream of MeCP2.

This research will allow us to determine how significant mutations in STK9 are in the development of RTT and in X-linked mental retardation. In addition, our studies will allow us to begin to dissect out the functional role or roles of STK9, its relation to MeCP2, and the mechanisms regulating both. Such knowledge of the biological processes involved will provide an important insight into the aetiologies of RTT.

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Charlotte Kilstrup-Nielsen, Ph.D.

University of Insubria (Italy)

Characterization of novel proteins influencing MeCP2 activity and analysis of their possible involvement in Rett Syndrome.

\$80,000

Lay Progress Report (November 2006)

Mutations in MeCP2 are known to cause most cases of Rett syndrome. MeCP2 is important for the proper function of specific brain cells and it is known to be able of binding a particular fraction of genomic DNA thereby regulating its function. However, the precise mechanisms of how MeCP2 exerts its function are only poorly understood and, in particular, it is not known how the activity of this protein is regulated. Moreover, several facts indicate that other factors may be involved in the onset of RTT: first of all, patients without defects in MeCP2 have been found and, second, two patients with the same mutation in MeCP2 can develop RTT with very different severity. In accordance with this mutations in a second gene, CDKL5, have recently been identified in some RTT patients. CDKL5 is a rather uncharacterized protein but the common clinical picture in patients with mutations in MeCP2 and CDKL5 suggest that the two proteins work in the same pathways. Importantly, CDKL5 seems to be involved not only in Rett syndrome but also in other cases of mental retardation and infantile spasms.

We believe that defects in genes working either in parallel with MeCP2 or directly regulating its activity may be important for the Rett syndrome. Therefore, we are characterizing some proteins that seem to be involved in regulating MeCP2. Our main focus has been to understand better the functions of CDKL5 and several of our results have confirmed the hypothesis that MeCP2 and CDKL5 work together; we have shown that CDKL5 is able to chemically modify not only itself but also MeCP2, probably an important mechanism in regulating its functions. These results suggest that mutations in CDKL5 cause Rett because MeCP2 is not properly regulated but we expect that CDKL5 may regulate not only MeCP2 but also have other functions. We have therefore analyzed some CDKL5 derivatives carrying Rett causing mutations and found that in some cases the mutated protein has lost its modifying capacity whereas in other cases it turns hyper-active. Even if these results need further confirmation they indicate that CDKL5 activity needs to be tightly regulated and that it might be important to search also for gene duplications in patients with infantile spasms and mental retardation.

Lay Summary

Rett Syndrome (RTT) is one of the leading causes of mental retardation in girls. In most cases, RTT is caused by

mutations in a protein called MeCP2, which is important for the proper function of specific brain cells. MeCP2 is able of binding a specific fraction of genomic DNA thereby regulating its function. However, the mechanisms of how MeCP2 exerts its function in the brain are only poorly understood, and in particular it is not known how the activity of this factor is regulated. Furthermore, several facts indicate that other factors may be involved in the onset of RTT: first of all, patients have been found without defects in MeCP2 and, second, two patients with the same mutation in MeCP2 can develop RTT with very different severity. We believe that defects in other genes, working in parallel with MeCP2 or directly regulating the activity of this protein, may be important for the disease. Therefore, we have performed experiments in our laboratory, which have led to the identification of five proteins that seem to be involved in regulating MeCP2 activity. With this proposal we intend to characterize these five proteins and in particular we will investigate if defects in MeCP2, such as those causing RTT, are regulated differently with respect to MeCP2 from healthy persons. We believe that the obtained results will facilitate the discovery of new factors involved in RTT, thus contributing to the development of diagnostic tools as well as therapeutic strategies.

Abstract

Rett Syndrome (RTT) is one of the leading causes of mental retardation and developmental regression in girls. The majority of cases of RTT are caused by mutations in the gene encoding the methyl-CpG-binding protein 2 (MeCP2), whose expression pattern in brain suggests a function for MeCP2 in regulating neuronal maturation and plasticity. MeCP2 binds methylated DNA and it is well established that it represses gene expression by modifying chromatin structure. However, the exact mechanisms of how MeCP2 regulates gene expression still have to be discovered; in particular recent findings suggest that MeCP2 function is regulated by post-translational modifications but the enzymatic activities involved in these modifications are not known. Furthermore, MeCP2 mutations have not been found in all patients, suggesting that other genes involved in the pathogenesis of RTT have to be discovered. We believe that the identification of the molecular mechanisms by which MeCP2 regulates gene expression and of other proteins, participating in the same pathway of gene repression by regulating MeCP2 function, will permit to better characterize RTT. We have identified five novel MeCP2 interacting proteins in a genetic screen and with this proposal we intend to characterize the functional role of these interactions. These five factors can be divided in two functional groups: proteins within the first group seem to be involved in the post-translational modification of MeCP2 whereas the other group contains proteins possibly containing transcriptional activity or chromatin modifying activities. We will characterize how these factors may be involved in influencing MeCP2 activity and set up functional assays to understand how these interactors may be involved in RTT onset.

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Genevieve Laforet, M.D., Ph.D.

University of Massachusetts

HDAC Activation in RTT

\$100,000

Lay Progress Report (August 2005)

Our research focuses on the underlying molecular mechanisms leading to abnormal brain development in Rett syndrome (RTT), with an eye toward correcting these mechanisms using drug therapy. RTT is caused by mutations in MeCP2, a protein involved in control of gene expression. We use mouse models that are deficient in MeCP2, as well as brain cells (neurons) isolated from these animals, to investigate the disruptions in cell function and gene expression associated with MeCP2 impairment. MeCP2 normally recruits other proteins called histone deacetylases (HDACs) that chemically modify histones, the protein scaffolds that house DNA in the nucleus. The resultant histone modifications

alter DNA-histone interactions and make genes in the DNA less accessible for expression. We have demonstrated that MeCP2 deficiency makes it harder for HDACs to make these important modifications, as reflected by reduced levels of histone deacetylation in MeCP2-deficient embryonic neurons in culture. This in turn perturbs expression of developmentally important genes such as *Dlx5* and *Sgce* in embryonic neurons. We have shown that drugs such as theophylline and anacardic acid can restore the proper balance of chemical modifications that are disrupted when MeCP2 and HDAC activity is disrupted, making them possible therapeutic candidates for RTT. In addition to work with cultured cells, we are also testing MeCP2 deficient mice to characterize their neurological and behavioral abnormalities, in order to devise practical experimental paradigms to evaluate drug treatments in RTT animal models. Based on these studies, we are beginning treatment trials to evaluate whether drugs like theophylline that modify HDAC activity can prevent or reduce symptoms in MeCP2 deficient mice. Our ultimate goal is to determine whether drugs that act on histone-modifying enzymes are useful therapeutic agents for correcting abnormal gene expression and restoring normal neuronal development in RTT animal models and patients.

Lay Summary

Much of the master plan for brain development is written in the DNA of brain cells. Specific stretches of DNA called genes spell out individual instructions that must be executed with exquisite temporal and spatial precision. Normal brain development depends critically on the orderly activation and silencing of different genes. Rett syndrome (RTT) is caused by defects in a protein called MeCP2 that is involved in this silencing process. MeCP2 participates in a cascade of events that governs gene turnoff. First it binds to specially tagged DNA sequences that indicate areas to be shut down. Then, it recruits and activates helper proteins called HDACs that modify the architecture of the DNA so it becomes locked away in an archival rather than active form. In RTT, defective MeCP2 is unable to execute this silencing mechanism. Because brain development requires such tight control of gene activity, faulty MeCP2 function leads to abnormal maturation in key brain regions, resulting in the predominantly neurological manifestations of RTT.

Our study uses this current understanding of how MeCP2 works to investigate potential therapies for RTT. Since MeCP2 impairment causes RTT, in principle it may be possible to treat RTT by artificially restoring some aspects of MeCP2 function. We know that one important role for MeCP2 is to recruit and activate HDAC helper proteins to lock down DNA for gene silencing. If there were some way to stimulate these HDACs directly, we could bypass the MeCP2 malfunction and potentially prevent or reduce the severity of RTT. Recent studies have demonstrated that the commonly used anti-asthma drug theophylline can stimulate HDAC activity. In our study, we will use mouse models of RTT to test whether theophylline can stimulate HDACs in brain and reduce the RTT-like symptoms in these animals. Testing this approach in mice provides a first step towards the development of useful mechanism-based therapies for RTT patients.

Abstract

Most cases of RTT are caused by mutations in the gene encoding MeCP2, a transcriptional repressor that is highly expressed in brain. MeCP2 acts by binding methylated CpG residues in genomic DNA and recruiting co-repressors and histone deacetylases (HDACs), leading to deacetylation of histones and induction of a transcriptionally silent chromatin conformation. According to this model, MeCP2 dysfunction in RTT causes failure of HDAC activation, abnormally elevated levels of histone acetylation, and inappropriate de-repression of gene transcription. This dysregulation leads, by mechanisms still under investigation, to maturational failure in key brain regions, resulting in the predominantly neurological RTT phenotype. Despite elucidation of the gene defect underlying RTT, no effective treatments have yet been developed. However, the MeCP2-HDAC recruitment model of RTT pathogenesis predicts the direct upregulation of HDAC activity might compensate for defective HDAC activation by MeCP2 and thereby abrogate the RTT phenotype. In our proposal, we will test whether the anti-asthma drug theophylline, which has shown to stimulate HDAC activity, can ameliorate the RTT-like phenotype of *Mecp2* null mice. Drug levels will be quantified by HPLC and correlated with HDAC stimulation in brain at different points in development. Once an optimal dosing regimen has been established, we will evaluate the effects of treatment in *Mecp2* deficient mice via cognitive and social behavioral testing and assessment of gait, activity, breathing patterns, body and brain weights, and neuropathology. We will also investigate the biochemical effects of theophylline in brain and cultured neurons using HDAC activity assays, Western analysis of histone acetylation/methylation, and biochemical assays of HDAC posttranslational modifications. This study holds promise for the development of mechanism-based therapeutics for RTT. In addition, the HDAC activating properties of theophylline provide a new molecular tool for probing the biochemical events involved in MeCP2- and HDAC-mediated transcriptional repression.

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Janine LaSalle, Ph.D.

University of California Davis

The Role of MeCP2 in the Ontogeny of Cerebral Cortical Neurons

\$50,000 (1 yr extension of existing award)

Lay Progress Report (August 2005)

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 deficient 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. This proposal used 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. Using this approach, a family of genes called the "ID" for "Inhibitors of Differentiation" were identified. The ID genes normally inhibit differentiation of cells, including neurons. The ID gene family showed abnormally high expression in the Mecp2 deficient mouse model and in Rett syndrome postmortem brain. These results suggest that increased expression of the ID genes may inhibit normal neuronal maturation, resulting in immature neurons and abnormal brain development in the Rett brain. These results could have relevance for future Rett treatments, as ID inhibitor drugs are currently being designed for potential cancer treatments.

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 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.

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. Aim 1 has been completed and a list of potential MeCP2 target genes identified. In the second aim of the proposal, genes with significantly altered expression levels in cells with blocked MeCP2 activity will be tested by chromatin immunoprecipitation (ChIP) to confirm that they are primary targets of MeCP2 and by quantitative RT-PCR to confirm expression differences. The candidate genes will then be 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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Sanford Leuba, Ph.D. & Jordanka Zlatanova, Ph.D.

University of Pittsburgh & Polytechnic University

Rett Syndrome: MeCP2 action in chromatin

\$100,000

Lay Progress Report (August 2005)

Mutations in the MeCP2 protein are associated with Rett Syndrome. MeCP2 binds methylated DNA, is associated with chromatin, is found in densely methylated regions of the genome and represses transcription. Our research aims to understand the mechanism of action of MeCP2 in chromatin and in transcription. A better understanding of the mechanism of MeCP2 should lead to a rational design of therapies to maintain normal functioning of MeCP2 even when the gene is mutated, and thus prevent the onset of Rett Syndrome in patients identified at risk for the disease or treat an already progressing disease.

We show that MeCP2 binds unusual DNA structures including nucleosomes in which the DNA is wrapped around core histone proteins and Holliday junction DNA, which is an intermediate in DNA recombination. We also show that a histone chaperone protein, nucleosome assembly protein 1 (NAP1), removes MECP2 on nucleosomes.

NAP1 is the first protein ever shown to displace MeCP2 from its natural binding site and suggests a mechanism of how MeCP2 binding to methylated nucleosomes may be regulated in vivo.

Lay Summary

Mutations in the MeCP2 protein are associated with Rett Syndrome. MeCP2 binds methylated DNA, is associated with chromatin, is found in densely methylated regions of the genome and represses transcription. Our proposal aims to understand the mechanism of action of MeCP2 in chromatin and in transcription. We will be using routine biochemical and innovative single-molecule approaches to understand how this protein effects chromatin structure and thus regulates transcription. Using single-pair fluorescence resonance energy transfer (spFRET), we will investigate the structural changes in nucleosomes caused by MeCP2 binding. In spFRET, two fluorescent dyes, a donor dye and an acceptor dye, are covalently attached to known sites within the molecules. The absorption and emission characteristics

of the two dyes are such as to allow transfer of energy from the donor dye to the acceptor dye only if the two dyes are within ~1 to ~8 nm of distance. Because the efficiency of energy transfer depends on the distance between the two dyes, small distance changes within this range can be distinguished. From these small distance changes recorded in real time we can understand the mechanism of MeCP2 at a temporal and spatial resolution unachievable thus far. We will also determine how this protein regulates transcription in nucleosomes. A better understanding of the mechanism of MeCP2 action should lead to a rational design of therapies to maintain normal functioning of MeCP2 even when the gene is mutated, and thus prevent the onset of Rett Syndrome in patients identified at risk for the disease or treat an already progressing disease.

Abstract

Mutations in the MeCP2 gene have been found in patients with Rett syndrome and have been causally linked to the etiology of the disease. MeCP2 specifically interacts with methylated CpGs, is chromatin-associated, is found in densely methylated regions of the genome, and represses transcription in vitro and in vivo. The overall objective of this proposal is to investigate the mode of action of MeCP2 on chromatin structure and on transcription. To that end, we will (i) determine binding affinities of MeCP2 to CpG sites at different locations in the nucleosome DNA, (ii) investigate possible competitive binding of MeCP2 and linker histones on unmethylated and methylated mononucleosomes and nucleosome arrays, (iii) test the hypothesis that Mecp2 binding to nucleosomes increases nucleosome strength, (iv) elucidate the effect of MeCP2 binding to different nucleosome sites on nucleosome dynamics (breathing and opening/closing), and (v) set up a single-pair fluorescence resonance energy transfer (spFRET) assay to follow nucleosome fate during chromatin transcription at high temporal and spatial resolution. The long-term objective (not achievable in two years) is to understand the mechanism of transcriptional repression by MeCP2. Our experiments are designed to determine the mechanism of MeCP2 binding to its chromatin target and how this binding contributes to altering chromatin structure and regulating gene expression. A better understanding of this dynamic mechanism can lead to rational design of therapies to maintain normal functioning of MeCP2 even when the gene is mutated, and thus prevent the onset of Rett Syndrome in patients identified at risk for the disease or treat an already progressing disease.

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Berge Minassian, M.D.

Hospital for Sick Children

Generation and characterization of mice with isoform-specific Mecp2-deficiency

\$100,000

Lay Progress Report (November 2006)

We reported in 2004 the discovery of a new form of the Mecp2 protein. This form, now called Mecp2-e1 is ten times more abundant in the brain than the previously known form Mecp2-e2. In order to analyze the role of each protein in Rett syndrome, we have aimed to generate two types of mice, one type lacking Mecp2-e1, and the other lacking Mecp2-e2. The genetic engineering required turned out to be extremely difficult, but we have now almost completed the process. We are running final diagnostics to ensure the constructs are correct. As soon as this is done, we will rapidly generate the two different mice, and analyze them behaviorally and pathologically. We will then be able to assign the role of each mec2 form in the different features of Rett syndrome.

Lay Summary

Until recently, the MECP2 gene was thought to have one form: four DNA sequence blocks (exons) coming together to form the gene, which then produces the MeCP2 protein. We discovered that three of these blocks (exons 1, 3 and 4) can come together leaving out exon 2 and form a somewhat different MECP2 gene and protein. Surprisingly this new form (MECP2_v2) is present in 10 times more amount to human brain than the previously known form (MECP2_v1). On the other hand, MECP2_v1 is evolutionarily more recent and seems to have evolved with mammals and is not present in non-mammalian vertebrate species. The great majority of patients with Rett syndrome have mutations in the large DNA blocks of exons 3 and 4 i.e. in the regions common to both forms.

Is the new, and more abundant, MeCP2_v2 protein the only form that is important in Rett syndrome, and the previously known form a mere evolutionary curiosity? Or has MeCP2_v1 evolved to serve a crucial purpose, e.g. in an important region of the brain, or at an important stage of brain development, or to carry out an important role specific to it? Answering these questions is of fundamental importance to the direction in which research on MeCP2 and Rett syndrome will need to be oriented. At this time the best way to answer this question is to remove each protein, separately, from mouse while keeping the other one intact and studying the effects of missing each protein. This is what we plan to do in this project. The mouse lines we generate will be made available to other Rett syndrome scientists. These mice will be an indispensable tool in uncovering the particular functions of each form of MeCP2. This is essential to the complete understanding of what is disturbed in brain cells in Rett syndrome and to how we can correct it.

Abstract

We discovered a splice variant of the MECP2 gene (MECP2_v2) encoding a previously unknown isoform of the MeCP2 protein. MeCP2_v2 possesses a distinctive amino-terminus composed of polyalanine and polyglycine repeats similar to the ERK1 signal transduction protein and to developmental transcriptional regulators belonging to the HOX and SOX gene families. We also showed that in human brain this MECP2 transcript is expressed in amounts 10-fold higher than the previously known variant (MECP2_v1). We found, in 1 of 500 Rett syndrome patients tested so far, a mutation that appears specific to MECP2_v2, though the effect of this mutation in brain cannot presently be tested. MECP2_v2 is present in numerous species and is therefore likely the ancestral form of the gene. MECP2_v1, on the other hand, appears to have evolved with mammals and absent in other vertebrates.

Three mouse models of Rett syndrome have been produced, and in all three the mutation is such that both Mecp2 isoforms are destroyed. With this proposal we aim to generate mouse models deficient separately of each isoform. We will characterize the phenotype of each mouse at the neurologic, behavioral, electrophysiologic and pathologic levels. This will allow us to determine the contribution of each of the two Mecp2 isoforms to the phenotype, which will be fundamental to the direction of future research on MeCP2 and in Rett syndrome. We will make these mice readily available to the Rett syndrome research community. Deficient purely of one or the other protein, these mice will serve as a crucial resource in unraveling the precise cellular function of each MeCP2 isoform towards a complete understanding of the biology underlying Rett syndrome.

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Sam Pfaff, Ph.D.

Salk Institute

Analysis of Neuronal Development, Axon guidance and Connectivity in MeCP2 mutant mice

\$50,000

Lay Progress Report (August 2005)

The methyl-CpG binding protein MeCP2 is thought to control gene expression epigenetically, which has made it difficult to predict what CNS functions are comprised in MeCP2 mutant cells. Therefore, the main objective of this proposal is to identify cellular processes that fail to occur properly in MeCP2 mouse mutants. We will focus on development of the CNS because human males deficient in MeCP2 exhibit profound defects at birth, and mouse genetic studies have helped to narrow the key functions of MeCP2 to post mitotic neurons. Two key aspects of CNS development will be examined: neuronal subtype specification and axon navigation. A better understanding of MeCP2's functions in CNS development should help to target the appropriate pathways to ameliorate the devastating affects of this genetic disease.

Our research objectives are directly related to two of the key objectives of the RSRF:

- 1) To characterize the role of MeCP2, including MeCP2-target genes in normal structure and function of the nervous system.
- 2) To describe the role of MeCP2 during normal brain development.

Lay Summary

During the last decade tremendous progress has been made in characterizing how the nervous system develops. In particular, the spinal cord has been used as a model for understanding how other more complex regions of the brain are specified and wired correctly. In this proposal we will use the spinal cord as a model system for examining CNS development at high resolution. The phenotypes associated with Rett syndrome are not expected to be restricted to the spinal cord, but the widespread expression of MeCP2 during embryonic development raises the possibility that this is one site where the functions of MeCP2 can be unmasked. Since the spinal cord shares many developmental features with other regions of the CNS, the clues we gain from these studies should help to focus our attention on the relevant processes that fail to occur properly in the absence of MeCP2 function.

Abstract

Although MeCP2 has been implicated as a negative-regulator of gene expression, our knowledge of the genes that become dysregulated following the loss of MeCP2-function remains fragmentary. In particular, it has been difficult to relate the epigenetic function of MeCP2 with the phenotypes associated with Rett syndrome. Since human males deficient in MeCP2 die around birth, it is predicted that MeCP2 has critical functions during embryonic development of the central nervous system. However, many of the studies of MeCP2 mouse mutants have focused primarily on complex or indirect measures of the nervous system such as animal behavior and survival. In this proposal we plan to investigate three core features associated with the development of the central nervous system: (1) neuronal subtype specification, (2) axon pathfinding, and (3) circuit formation. If defects in one or more of these processes are found in MeCP2 mutant mouse embryos, this should help to identify cellular and molecular pathways that need to be targeted to ameliorate the affects of Rett syndrome.

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Fyodor Urnov, Ph.D. & Egor Prokhorchouk, Ph.D.

Sangamo BioSciences & Institute of Gene Biology, Moscow

Use of designed zinc finger protein nucleases in gene correction therapy for Rett Syndrome

\$100,000

Lay Progress Report (August 2005)

Scientists at Sangamo are working to build proteins that would work as "genome editors" to directly correct the disease-causing mutation in RTT patients. Sixteen such proteins have been assembled; test-tube experiments have shown that one such protein is of very high quality, whereas other proteins require optimization that will be performed over the next 6-8 months. Scientists at the Centre for Bioengineering are creating a "humanized mouse" - an animal that carries the human MeCP2 gene disabled by the RTT mutation and that could be used to test the "genome editor" proteins.

Lay Summary

Many human diseases, including hemophilia, sickle-cell anemia and Rett syndrome, are caused by mutation errors in the genetic material (DNA) of the patient. It may seem at first that the simplest cure for these diseases is to simply provide the patient with some error-free DNA. This approach is known as "gene therapy" and turned out to be quite difficult to perform successfully: it turned out that human cells reject this error-free DNA, and even when they accept it, it fails to function normally, or is dangerous for the cell and can cause cancer. This proposal describes a new technology that overcomes these obstacles: gene correction. Gene correction invokes the cell's own machinery to repair the error in the cell's own DNA. This remarkable feat is accomplished by breaking the DNA precisely where the error occurs using a molecular machine that has been engineered for this purpose (this machine is known as a "zinc finger chimeric nuclease"). To heal this break, the cell copy-pastes some genetic information from a different DNA molecule that the scientists supply to it, a DNA molecule that does not contain the error. To use gene correction in therapy of Rett syndrome, one must first show that correction of this error in an animal will prevent symptoms from occurring, or alleviates some symptoms. We propose to use genetic engineering to create such animals and to investigate if "gene correction" of the mutation is effective in that regard. This approach will not provide a cure, but there is every reason to hope that at least some of the symptoms can be made less severe.

Abstract

The present proposal describes studies aimed at developing "gene correction" therapy for RTT. It is unlikely that gene correction will provide a cure for RTT, but it is entirely reasonable to expect that restoration of wild-type MeCP2 function to a large cell population will alleviate a subset of disease symptoms. High-frequency homologous recombination (HR) in mammalian cells can be achieved by invoking the cell's own machinery for double-strand break (DSB) repair (Valerie and Povirk *Oncogene* 22: 5792). The established way for targeting such a DSB is via the fusion of a zinc finger-protein based designed DNA binding domain to an endonuclease domain (Wilson *Nat Biotech.* 7:759). We propose to generate a panel of mouse ES lines carrying MeCP2 disabled by the most common mutations that occur in RTT patients, and to design a panel of chimeric ZFP-nucleases (Jamieson et al. *Nat. Rev. Drug Disc.* 2:361) specific for these mutated loci. Such nucleases have been shown to massively potentiate HR into reporter loci in mammalian cells (e.g. Porteus and Baltimore *Science* 300:5620), and function very robustly at endogenous loci, yielding targeting frequencies fully compatible with the notion that "gene correction" will be therapeutically effective. Feasibility of ZFP nuclease-driven gene correction as a therapeutic option for RTT will be evaluated by ZFP nuclease-driven targeting in ES cells, neurons differentiated ex vivo from these ES cells, and in female animals heterozygous for the mutations. These experiments, if successful, can serve as the basis for a formal preclinical animal safety and efficacy study using the reagents and animal models we have generated. The transition from animal data to human clinical trials is expected to be greatly facilitated by high conservation of mouse and human MeCP2, which will allow to port the reagents and protocols developed in the present research effort with animal modification.

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Post-Doctoral Fellowship Awards

Skirmantas Kriaucionis

University of Edinburgh

Mentor: Adrian Bird

Investigation of Mitochondrial Defects in a mouse model of RTT

\$100,000

Lay Progress Report (August 2005)

The MECP2 gene is often mutated in cases of the neurological disorder Rett Syndrome. The major suggested function of MeCP2 is to make some genes silent, that is to prevent them from producing proteins. It is likely that the cause of Rett Syndrome is the failure of mutated MeCP2 to repress its target genes. We initiated a screen for MeCP2 target genes and identified a group of genes in the brain of a mouse model of Rett Syndrome. Two of these genes which were mis-regulated belong to the mitochondrial respiration chain. Mitochondria are found within cells, and are responsible for the production of ATP, which is the main source of energy. The failure to efficiently produce energy might compromise energy-demanding cells such as neurons in the brain. While producing energy, mitochondria consume oxygen. We examined the mitochondrial function of by measuring oxygen consumption in the mouse model of Rett Syndrome and detected increased and inefficient respiration. Interestingly, abnormal oxygen consumption was detected when mice acquire Rett Syndrome symptoms. Additionally, we examined mitochondrial function by separating components of the respiratory chain and measuring their activities. Finally to get a better insight into the MeCP2 role in silencing genes, we created and tested a construct which allows the regulation of MeCP2 presence/absence using small molecules.

Lay Summary

The brain, and in particular its nerve cells, consume a large amount of energy in the form of ATP. ATP is synthesized in sub-cellular organelles called mitochondria, which are sometimes referred to as "the powerhouses of the cell". A number of neurological and neuro-muscular diseases have been traced back to defects in mitochondria. While looking for alterations in gene expression in Mecp2-null mice – a model for Rett Syndrome – we noted mis-expression of genes that encode key mitochondrial components. Follow-up experiments showed that brain mitochondrial function, as measured by consumption of oxygen under a variety of conditions, is abnormal in these mice. Our proposal is to ask what has gone wrong with these mitochondria. Is the problem only seen when symptoms start or before that? Are mitochondria outside the brain normal or not? What is the connection between deficiency of MeCP2 and mitochondrial dysfunction? Does lack of MeCP2 affect mitochondrial function quite directly, or are there many steps in between? Finally, we wish to know the extent to which mitochondrial abnormalities might contribute to the symptoms of Rett Syndrome.

Abstract

We subjected mRNA from Mecp2-null mouse brains to global analysis of gene expression using a variant of differential display called ADDER. The analysis revealed that genes for two components of the mitochondrial respiratory chain are mis-regulated in the mutants. We therefore hypothesized that the phenotype of these mice might involve mitochondrial abnormalities. In collaboration with Dr. Nikki MacLeod's laboratory (Edinburgh University) we investigated mitochondrial respiration in Mecp2-null mouse brain using oxygen electrode. The results consistently showed abnormal respiration in brain mitochondria from mutant mice compared to wildtype controls. Potential involvement of mitochondrial malfunction in Rett Syndrome has been considered previously, and mitochondrial abnormalities have been noted in muscle biopsies

of Rett patients. We are interested to investigate further the molecular causes and consequences of defective mitochondria in MeCP2-deficient mice. We propose to:

- a) investigate mitochondrial respiration during progression of symptoms and in different tissues;
- b) examine the ultrastructure of mitochondria by electron microscopy;
- c) investigate the abundance and integrity of different respiratory complexes;
- d) investigate the possibility of direct MeCP2 involvement in regulation of mitochondrial gene expression.

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Mizue Hisano, Ph.D.

Lawrence Berkeley National Lab

Mentor: Terumi Kohwi-Shigematsu

Characterization of MeCP2-target genes in RTT

\$100,000

Lay Final Report (November 2006)

This post-doctoral fellowship was transferred from Mizue Hisano to Masaru Miyano.

We studied higher-order chromatin structure at the Dlx5/6 locus and found change in the chromatin looping status in the Mecp2-null brain. We are in the process of examining the effect of MECP2 ablation on expression of the Dlx gene family. We have further examined the expression status of downstream candidate genes of Dlx5, and have identified Rxrg and Crabp1 to be dysregulated in the frontal cortex of Mecp2-null brains. During the past two years, we have addressed the potential effect of Mecp2 mutation on muscle regeneration. Muscle rigidity and reduced muscle mass are both known as typical symptoms of RTT patients. We focused on adult muscle progenitor cells, satellite cells, that are thought to represent the only population of committed myogenic progenitors in postnatal myogenesis. The result of this study suggests that muscle regeneration is impaired for Mecp2-null satellite cells which exhibit a defect in the myotube formation. In adult Mecp2-null mice, substantially reduced numbers of activated satellite cells were detected upon injury when compared to wild-type mice. We have several candidate genes, regulated by MECP2, that are responsible for these phenomena. Our data indicating that MECP2 plays a key role in satellite cell function predicts impaired postnatal myogenesis in Rett syndrome.

Lay Summary

Mutations in MECP2 have been identified in Rett syndrome (RTT), a childhood neurological disorder. MeCP2 binds to methylated DNA and represses transcription of a multitude of genes. Therefore, defects in MeCP2 may affect the temporal and spatial expression of many key proteins in neurons, resulting in malfunction of the central nervous system

(CNS). In order to understand how mutated MeCP2 causes the neurological disorder, it is crucial to identify which genes are dysregulated in the MeCP2-deficient mouse brains. We have identified a gene closely associated with the forebrain neurons that use γ -aminobutyric acid (GABA) as their neurotransmitter, a chemical released by a neuron upon stimulation and activates another neuron. This gene induces these neurons to express the glutamic acid decarboxylase (Gad), enzymes that synthesize GABA from glutamic acid. In wild type mouse brain, this gene is located near an MeCP2-bound genomic DNA sequence in vivo. In MeCP2-deficient mouse brain, this gene is expressed consistently at a higher level than in wild-type mouse brain. We propose to test whether the increased level of this gene is sufficient to cause some of the neurological phenotypes, by establishing mouse lines by introducing additional copies of this gene so that this gene will be over-expressed to a level similar to that found in MeCP2-deficient mouse brains. To search for additional target genes for MeCP2, we will examine expression of a series of genes located in close proximity to genomic sequences cloned as MeCP2-bound sequences in vivo. This group of genes includes several neuron-specific genes implicated in ataxia, seizures, and delayed CNS development. We will examine if any of these candidate genes shows changes in expression in MeCP2-deficient mouse brains compared to wild type mouse brains. Identification of neuronal genes that are direct targets of MeCP2 is important for understanding the RTT pathology and for future therapeutic applications for RTT treatment.

Abstract

Mutations in MECP2 have been identified in Rett syndrome (RTT), a childhood neurological disorder. MeCP2 binds to methylated DNA and represses transcription of a multitude of genes. Therefore, defects in MeCP2 may affect the temporal and spatial expression of many key proteins in neurons, resulting in malfunction of the central nervous system (CNS). In order to understand how mutated MeCP2 causes the neurological disorder, it is crucial to identify which genes are dysregulated in the MeCP2-deficient mouse brains. We have identified a gene closely associated with the forebrain neurons that use γ -aminobutyric acid (GABA) as their neurotransmitter. This gene induces these neurons to express the glutamic acid decarboxylase (Gad), enzymes that synthesize GABA from glutamic acid. In wild type mouse brain, this gene is located near an MeCP2-bound genomic DNA sequence in vivo. In MeCP2-deficient mouse brain, this gene was found to be expressed consistently at a higher level than in wild-type mouse brain. We propose to test whether the increased level of this gene is sufficient to cause some of the neurological phenotypes, by establishing transgenic mouse lines overexpressing this gene to a level similar to that found in MeCP2-deficient mouse brains. To search for additional target genes for MeCP2, we will examine expression of a series of genes located in close proximity to genomic sequences cloned as MeCP2-bound sequences in vivo. This group of genes includes several neuron-specific genes implicated in ataxia, seizures, and delayed CNS development. We will examine if any of these candidate genes shows changes in expression in MeCP2-deficient mouse brains compared to wild type mouse brains. Identification of neuronal genes that are direct targets of MeCP2 is not only important for understanding the pathology of RTT but also for future therapeutic applications for RTT treatment.

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Satoko Matsumura, D.D.S., Ph.D.

New York University

Mentor: Angus Wilson

Uncovering a role for MeCP2 as an activator of gene expression

\$97,808

Lay Progress Report (August 2005)

It is well established that mutations in MeCP2 protein contribute to the neurological symptoms associated with Rett Syndrome (RTT) in young women. Considerable effort has been devoted to understanding the function of MeCP2 at both the cellular and molecular level. With the generous support of the RSRF, I have been able to show using various biochemical approaches that the MeCP2 protein interacts with DMAP1, a subunit of the so-called "maintenance CpG methylase" enzyme complex. This enzyme is responsible for copying patterns of DNA methylation when cells divide or repair parts of the chromosome that have been damaged. These methylated CpGs act as landing sites for MeCP2, which in turn controls the expression of nearby genes. It is likely that RTT results from subtle changes in the patterns of methylation or ability of MeCP2 to be recruited to methylated DNA. My current studies will focus on the functional consequences of the MeCP2-DMAP1 interaction and the important question of whether altered DMAP1 function contributes to RTT.

Lay Summary

Rett Syndrome is a devastating neurodevelopmental disorder affecting mostly girls. The vast majority of cases arise from mutations in a single gene known as MeCP2, which codes for a protein involved in the control of other genes. Although the discovery of the genetic lesion is a major breakthrough there are still many important questions to be answered before effective therapies can be designed. MeCP2 protein is made in almost every type of cell but the disease has its most severe effects in nerve cells of the brain. Careful studies to identify genes that are incorrectly controlled when MeCP2 is mutated have not uncovered any striking changes. It is thought that MeCP2 mainly functions to turn genes off but new evidence, including unpublished findings from our lab, suggests a limited role in turning genes on. We have shown that the binding of MeCP2 to a viral protein favors activation rather than repression. In the proposed project I will determine the mechanism by which viral protein reprograms MeCP2 and use this knowledge to search for human proteins that perform a similar function. The long-term goal of these studies is to find genes that need MeCP2 for the activity or special circumstances where MeCP2 inhibition is reduced.

Abstract

Our laboratory has been studying the molecular pathogenesis of Kaposi's sarcoma-associated herpesvirus (KSHV), focusing on the pivotal role of the latency-associated nuclear antigen (LANA). This protein tethers the viral genome to host cell chromatin and also regulates gene expression. Both functions require a short chromatin-binding motif in LANA that recognizes methyl-CpG protein 2 (MeCP2), the gene mutated in Rett syndrome. A striking observation is that association with LANA allows MeCP2 to function as an activator protein. The goals of this project are to characterize the MeCP2-LANA interaction in detail and use this knowledge to identify cellular polypeptides that interact in a similar manner and perhaps also utilize MeCP2 for activation rather than repression. These studies may uncover genes that utilize MeCP2 for their expression and whose dysregulation contributes to the pathophysiology of this devastating disease.

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Jean-Christophe Roux, Ph.D.

CNRS France

Mentor: Gerard Hilaire

Alterations of Respiratory Rhythm and Bioamine Metabolism in MECP2 Deficient Mice

\$100,000

Lay Summary

Rett Syndrome is a severe neurological disorder which could account for up to 10% of severe mental retardation of genetic origin in women. The clinical course of the disease consists of normal development during the neonatal period until 6 Æ 18 months followed by a number of clinical signs indicative of neurodevelopmental defects such as an arrest of brain development, a regression of acquisitions, and behavioural troubles with stereotypic hand movements. One of the most life-threatening symptoms of Rett Syndrome is the presence of severe anomalies of the respiratory rhythm which could potentially contribute to the frequent sudden deaths observed in this syndrome. Numerous hypotheses have been proposed to explain these respiratory alterations; among which a defect of brain maturation with alteration of endogenous bioaminergic metabolism (catecholamines and serotonin). Because mutations in a specific gene (MECP2) have been identified in most Rett patients, a mouse experimental model has been recently created by genetic invalidation of this gene and the mutant *Mecp2*-deficient mice present a number of clinical symptoms observed in Rett patients (Guy et al, Nature 2001). Because respiratory anomalies have been briefly reported in these mice, we performed a first set of experiments to record their breathing pattern. Our preliminary results confirmed respiratory alterations in adult *Mecp2*-deficient mice and in addition revealed bioaminergic metabolism alterations (submitted J. Physiol.). We hypothesize that *Mecp2* deficiency first induces bioaminergic alterations which in turn lead to respiratory deficits. Therefore, we propose to thoroughly analyze the development from birth to adulthood of the respiratory and bioaminergic systems in these mutant mice and to check whether the bioaminergic alterations precede the respiratory ones and whether some pharmacological treatments could prevent or alleviate bioaminergic alterations and the resulting respiratory deficits in mice. In conclusion, as Rett patients have MECP2 mutations, respiratory alterations and possible bioaminergic alterations, our project in mice is particularly relevant to improve our understanding of Rett syndrome and to suggest some putative pharmacological treatments to soften respiratory deficits of Rett patients.

Abstract

Rett syndrome is a severe neurological disorder in which most of the patients have MECP2 gene mutations, life-threatening respiratory alterations and possible bioaminergic deficits. As our preliminary experiments in *Mecp2*-deficient mice suggested that inactivation of this gene in mice induced both bioaminergic and respiratory deficits (submitted to J. Physiol), we plan to perform the following 4 different experimental steps to study the:

- 1) development of respiratory troubles in awake *Mecp2*-deficient mice from birth to adulthood using plethysmography to record their breathing pattern (Step 1: from September 04 to march 05),
- 2) development of bioaminergic deficits in *Mecp2*-deficient mice from birth to adulthood using high pressure liquid chromatography and immuno-histochemistry (Step 2: from Sept. 04 to October 05),
- 3) mechanisms underlying the onset of the respiratory deficits via in vitro and in vivo approaches in neonatal and adult *Mecp2*-deficient mice (Step 3: from November 05 to April 06),
- 4) possibility to alleviate the respiratory deficits in adult *Mecp2*-deficient-mice by pharmacological treatments targeting the bioaminergic alterations (Step 4: from May 06 to September 06).

Our aims are to check whether the bioaminergic deficits precede and induce the respiratory ones (steps 1 and 2) and whether they may be prevented or alleviated by pharmacological treatments (steps 3 and 4).

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Matthew Tudor, Ph.D.

Whitehead Institute

Mentor: Rudolf Jaenisch

Genetic and molecular characterisation of the 3 MBD family members, MBD1, MBD2 and MeCP2

\$100,000

Recipient of the Alan P. Wolffe Memorial Fellowship

Lay Progress Report (August 2005)

This post-doctoral fellowship was transferred from Matthew Tudor to Qiang Chang.

I have been using the *Mecp2* mutant mice as model system to study the molecular mechanism of the Rett Syndrome (RTT). Our previous work has shown that the MeCP2 protein binds to the promoter of the BDNF gene (brain-derived-neurotrophic-factor) and can regulate its transcription in cultured neonatal neurons. However, it is not clear what role, if any, BDNF plays in vivo in RTT pathogenesis. In order to address this question, I have manipulated the level of BDNF in post-mitotic neurons in the postnatal brain in *Mecp2* mutant mice in a temporally- and spatially-controlled manner. My results clearly show that overexpressing BDNF in the postnatal brain of *Mecp2* mutant mice slows down RTT disease progression, whereas removing BDNF in the same neurons accelerates RTT disease progression. These results implicate BDNF and other components of the BDNF signaling pathway as potential candidates for treating RTT patients. In addition to studying the role of BDNF in RTT pathogenesis, I have been searching for more MeCP2 target genes. Research along this line will help us fully understand how mutations in the *Mecp2* gene cause RTT. Moreover, in collaboration with the laboratory of Dr. Sacha Nelson at the Brandeis University, I have identified a specific electrophysiological defect in the somatosensory cortex of the *Mecp2* mutant mice. This assay will be a valuable tool to study RTT at the synaptic level.

Lay Summary

In addition to MECP2, the gene responsible for Rett Syndrome, there are two other genes which are thought to have similar activities. In order to better understand how these three proteins interact with each other and with the genome we will study the family of proteins in mice. We propose a set of experiments that will answer the following questions:

- 1) Since all three family members bind to genetic material, which genes are bound by each of the proteins? Moreover, how does the pattern of binding change when one of the family members is missing, as is the case in Rett Syndrome?
- 2) What can we learn from mice that have mutations in all three genes? Surprisingly, such animals are no worse off than animals with mutations only in *Mecp2*. How can these observations be explained in view of current knowledge of the biochemical pathway in which they act?

We have developed unique techniques to answer these questions, and are on the verge of obtaining our first data. This work should significantly increase biologists' understanding of what these proteins do under normal circumstances as well as in the disease state, and possibly provide leads to therapies.

Abstract

MECP2, the gene mutated in Rett Syndrome, is a member of a family of proteins, all of which have been implicated in methyl-cytosine binding and histone deacetylation. We propose a series of experiments in the mouse which endeavor to characterize this family genetically and biochemically. We have generated mutations in each of the family members and propose the analysis of single and compound mutants. We will use our genetic reagents along with novel microarray-based technologies to obtain genome-wide profiles of the distribution of these proteins on chromatin. Additionally, we will characterize mice mutant for all known methyl-CpG-specific binding factors in order to determine whether methylated cytosines in these cells are in fact unbound by any activity, or whether we can uncover evidence that yet other functionally related factors exist. Results should be available relatively soon as all of the reagents for the proposed experiments are already in hand or soon will be. Single and compound mutant mice have already been generated, and the location analysis protocol has been validated on a pilot scale and will be ready for full-scale experimentation within

several weeks to a month.

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Juan Young, Ph.D.

Baylor College of Medicine

Mentor: Huda Zoghbi

Exploring the reversibility of the RTT phenotype: conditional restoration of MeCP2 in a mouse model of RTT

\$25,000 (6 month extension of existing award)

Lay Summary

Animal models of genetic diseases have proved to be invaluable in testing therapeutic approaches. The discovery of MECP2 as the gene mutated in Rett syndrome (RTT), and the subsequent generation of RTT mouse models, have provided the tools to investigate therapeutic strategies that could be applied to treat this severely disabling disease. Some unique features of RTT provide an additional stimulus to probe potential therapeutic approaches. The initial period of normal development and the absence of observable neurodegeneration are the best hope that with proper intervention the disease can be halted and reversed. An important precedent has been set: introduction of Mecp2 protein into neurons of mutant mice that do not produce Mecp2 rescues their symptoms. We are going to test the idea that the initial period of normal development displayed by RTT patients indicate that neuronal impairment has not occurred in this pre-regression stage or at least not to a degree that function cannot be restored. Thus, we propose to induce expression of a functional MeCP2 protein in a mouse model of RTT at different stages of disease progression. This will allow us to address the question of prevention or reversibility of the disease.

Abstract

The hypothesis of this proposal is that the progression of Rett syndrome (RTT) can be halted or reversed by restoring MeCP2 function at an appropriate time. Support for this hypothesis comes from the discovery that MeCP2 expression is not essential in the developing brain and that the expression of a Mecp2 transgene postmitotically can rescue the phenotypes of null Mecp2 mutants. I propose to evaluate if all the disease phenotypes can be reversed by expressing a MeCP2 transgene, and if there is a time-frame in which they should be rescued. I will also evaluate whether the two splice isoforms of MeCP2 (that differ in their abundance in brain) are capable of rescue. We know that a null allele can be rescued, but the majority of RTT causing alleles produce a defective protein. Therefore, I will also determine whether different mutant alleles of MeCP2 that still produce a protein are equally susceptible to being rescued by expression of a MeCP2 transgene. 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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