Rett Syndrome: A Prototypical Neurodevelopmental Disorder

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Rett syndrome, one of the leading causes of mental retardation and developmental regression in girls, is the first pervasive developmental disorder with a known genetic cause. The majority of cases of sporadic Rett syndrome are caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). MeCP2 binds methylated DNA and likely regulates gene expression and chromatin structure. Genotype/phenotype analysis revealed that the phenotypic spectrum of *MECP2* mutations in humans is broader than initially suspected: Mutations have been discovered in Rett syndrome variants, mentally retarded males, and autistic children. A variety of in vivo and in vitro models has been developed that allow analysis of MeCP2 function and pathogenic studies of Rett syndrome. Because the neuropathology of Rett syndrome shares certain features with other neurodevelopmental disorders, a common pathogenic process may underlie these disorders. Thus, Rett syndrome is a prototype for the genetic, molecular, and neurobiological analysis of neurodevelopmental disorders. NEUROSCIENTIST 10(2):118–128, 2004. DOI: 10.1177/1073858403260995

Introduction

Psychosocial development occurs along a well-defined trajectory in which specific milestones are realized at defined intervals. During development, two abnormalities can occur: developmental delay (i.e., the failure to meet milestones within a normal time frame) or developmental regression (i.e., the loss of previously acquired milestones). The latter is an ominous symptom of an underlying progressive neurological abnormality. Recent genetic and molecular advances have provided insight into a cause of developmental regression in girls: Rett syndrome.

Andreas Rett, an Austrian physician, originally described Rett syndrome in 1966 when he observed two girls with a similar abnormality in his waiting room (Rett 1966). The condition was generally unrecognized until Hagberg and others (1983) described a series of 35 European girls with Rett syndrome. Subsequent research found the prevalence of Rett syndrome to range from 1:10,000 to 1:22,000 (Percy 2002).

Classic Rett syndrome is a clinical diagnosis based on defined criteria (Hagberg and others 1985). Girls with

Rett syndrome are born after a normal pregnancy and uneventful delivery and have apparently normal development throughout the first 6 months of life. The head size is normal at birth, but a subsequent deceleration of head growth occurs after 2 to 4 months of life, ultimately resulting in an acquired microcephaly. Replacement of purposeful hand use with stereotyped movements occurs after 6 months of life. The hand stereotypies consist of midline hand wringing, clapping, and hand mouthing. Autistic features such as social withdrawal and impaired language are noted. A characteristic gait apraxia is prevalent, often curtailing or, in some cases, eliminating ambulation.

Clinical variants of Rett syndrome that do not completely meet the accepted diagnostic criteria have been described (Hagberg and Skjeldal 1994). At the severe end of the spectrum of atypical Rett variants are patients without a period of normal development, known as congenital variants of Rett syndrome. In contrast, mild forms exist that are known as forme fruste, or "worn down," variants. The preserved speech variant (PSV) is an interesting variant with particular unique characteristics, such as obesity and preserved head size (Zappella and others 1998).

Although girls with Rett syndrome are small for their ages, their brains are disproportionately small (Armstrong and others 1999). This small brain and grey matter atrophy led initially to the belief that Rett syndrome was a neurodegenerative condition of childhood, but the lack of progressive atrophy as the girls aged argued against a degenerative process. Neuropathological analysis of brains indicates no gross developmental abnormalities and no neuronal degeneration but rather small, closely

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packed neurons with reduced dendritic spines and arbors (Armstrong and others 1995).

Molecular Basis of Rett Syndrome

The genetic identification of the chromosomal region involved in Rett syndrome was initially hampered by the relative lack of familial Rett syndrome cases, which prevented a standard genetic linkage analysis. However, the syndrome is generally only found in girls; thus, it was assumed to be an X-linked dominant trait that caused fetal demise in males (Hagberg and others 1983). The small number of familial cases allowed exclusion mapping of the X chromosome, which narrowed the region to Xq27-qter (see references in Shahbazian and Zoghbi 2002). Candidate transcripts in the region were analyzed for variations, and ultimately mutations in methyl-CpG-binding protein 2 (*MECP2*), located at Xq28, were identified in Rett patients (Amir and others 1999). Further supporting the role of *MECP2* in Rett syndrome was the discovery that *MECP2* mutations were found in 80% of sporadic, classic Rett syndrome cases (Shahbazian and Zoghbi 2001). In these sporadic cases, most mutations arise from the parental germline and often occur at CpG mutational hotspots by deamination of methylated cytosine, which creates C-T transitions. Nearly 70% of the mutations in Rett syndrome arise from C-T transitions at eight CpG dinucleotides (Lee and others 2001).

MeCP2 and Other Methyl-CpG-binding Domain (MBD) Proteins

MeCP2 is the first protein identified that defines a family of methyl-CpG-binding proteins. Other identified members include methyl-CpG-binding proteins 1–4 (MBD1–4), all of which share an MBD (Hendrich and Bird 1998). MeCP2 contains three functionally defined domains: an amino-terminal MBD (Lewis and others 1992; Nan and others 1993), a transcriptional repression domain (TRD) (Nan and others 1997), and a carboxy-terminal domain (Chandler and others 1999). In addition, located within the TRD is a nuclear localization sequence (NLS) (Nan and others 1996). In experimental models, MeCP2 binds to single symmetrically methylated CpG dinucleotides via the MBD (Lewis and others 1992; Nan and others 1993) and interacts with corepressor complexes via the TRD (Nan and others 1997). The Sin3A corepressor complex, containing histone deacetylases 1 and 2, is one complex shown to interact with the TRD (see Fig. 2A) (Nan and others 1998). The histone deacetylases remove acetyl groups from histones, creating a compact form of chromatin that represses gene expression (see Fig. 1). On chromosomes, MeCP2 binding is densest in heterochromatic regions, but it is also present in euchromatic areas, identical to the distribution of 5-methyl cytosine. The carboxy-terminal domain appears to be important in facilitating binding of the protein to naked and nucleosomal DNA (Chandler and others 1999). MBD 1, 2, and 3 contain MBD and functional TRDs (Wade 2001), but MBD4 does not contain a
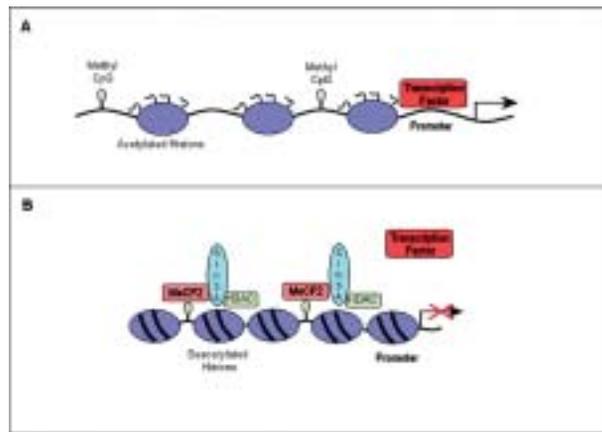

Fig. 1. Proposed molecular role for methyl-CpG-binding protein 2 (MeCP2). *A*, Illustrates DNA upstream from a promoter site. In this illustration, MeCP2 is not bound to methylated DNA. Thus, histones, represented by blue ellipses, are acetylated. In this state, the DNA is in an "open" conformation, allowing transcription factors (diagrammed as a red box) to bind to DNA and initiate transcription. When MeCP2 binds to the methylated CpG sites in DNA (*B*), Sin3A and histone deacetylases (HDACs) are locally recruited. HDACs deacetylate histones, inducing a "closed" DNA conformation that prevents transcription factors from binding to promoter sites, thus inhibiting gene transcription.

TRD and likely functions in nucleotide mismatch repair (Millar and others 2002). MBD 1, 2, and 4 are able to bind methylated DNA in vitro, whereas MBD3 cannot (Hendrich and Tweedie 2003).

Genotype/Phenotype Analysis in Rett Syndrome

Girls with Rett Syndrome

A number of genotype/phenotype comparisons have been performed in classic Rett syndrome (see refs in Shahbazian and Zoghbi 2002), but the analysis is complicated by X chromosome inactivation (XCI). Favorable skewing of XCI patterns in a mother with mutation in *MECP2* can allow a normal phenotype, whereas in the daughter, a balanced inactivation results in Rett syndrome (Amir and others 2000). Two studies found that truncating mutations are more severe than missense mutations, with early truncations more severe than late truncations (Cheadle and others 2000; Monros and others 2001). In contrast, other studies found no correlation between mutation type and severity (Amir and others 2000; Bienvenu and others 2000; Huppke and others 2000; Giunti and others 2001; Yamada and others 2001; Chae and others 2002). Amir and others (2000), in a study of girls with peripheral balanced XCI, found no correlation between mutation and clinical severity score; however, they identified increased respiratory problems and increased CSF homovanillic acid in truncating mutations and increased scoliosis in missense mutations.
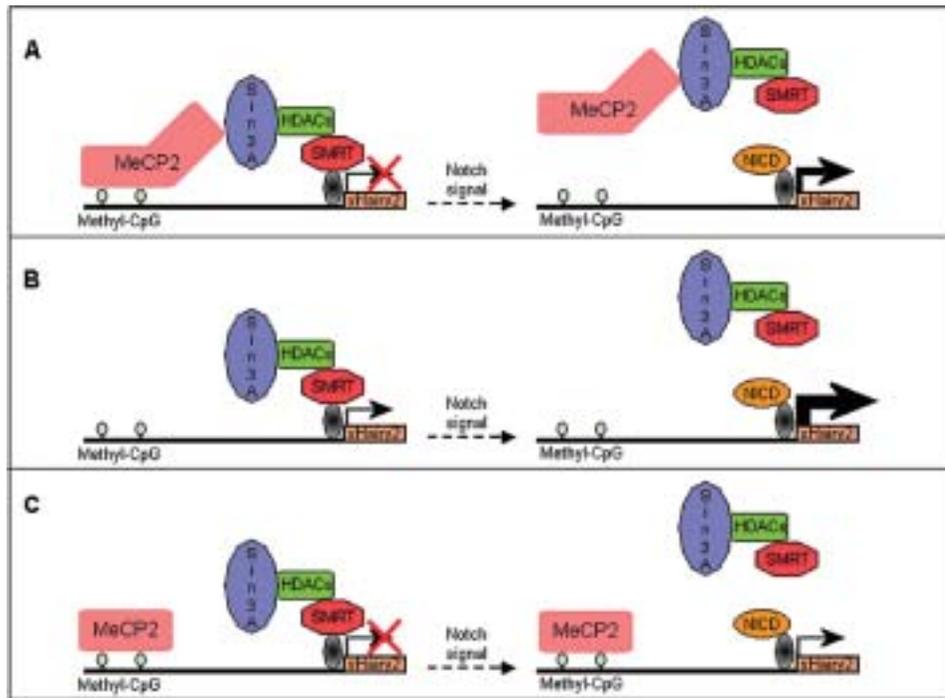
Fig. 2. Protein interactions on the xHairy2a promoter. On the left of panel *A*, methyl-CpG-binding protein 2 (MeCP2) is shown interacting with methyl-CpG groups on DNA in the xHairy2 promoter via the methyl-CpG-binding domain (MBD). MeCP2 interacts with Sin3A via the transcriptional repression domain (TRD): Sin3A acts as a bridge to connect MeCP2 to histone deacetylases (HDAC) and SMRT. SMRT interacts with proteins near the xHairy2 transcription start site and represses transcription. Notch signaling allows the nuclear accumulation of Notch intracellular domain (NICD), which interacts with the proteins near the xHairy2a transcriptional start site. This interaction displaces SMRT and induces xHairy2a expression (see right panel in *A*). Furthermore, the displacement of SMRT also induces displacement of MeCP2 from xHairy2a promoter. This displacement is dependent on the molecular bridge created between MeCP2 and SMRT by Sin3A. The removal of the MeCP2/Sin3A/SMRT complex allows normal expression of xHairy2a (right side of *A*). *B*, Demonstrates the expression of xHairy2a and molecular interactions seen in the absence of MeCP2. Even in the absence of Notch signal (left panel of *B*), xHairy2a expression is seen, in contrast to the transcription repression when MeCP2 is present (*A*). Notch signal displaces SMRT and results in greater xHairy2a expression (right side of panel *B*) than that seen when MeCP2 is present (right side of panel *B*). This results in decreased neuronal precursor cells compared to the normal situation depicted in *A*. *C*, Demonstrates the molecular interactions found when a mutant form of MeCP2, such as R168X, is expressed. This mutation creates a truncated form of MeCP2 that lacks the TRD, and thus no physical interaction is formed between MeCP2 and SMRT. Thus, when NICD displaces SMRT from the promoter, MeCP2 MBD remains bound to methylated DNA on the promoter. In this situation, xHairy2a expression occurs at a lower level than that seen when MeCP2 is completely displaced from the promoter (right panel of *A*). This decreased expression of xHairy2a after Notch signaling results in increased neuronal precursor cells. This figure was adapted from Stancheva and others (2003).

Recent work demonstrates a relationship between the clinical phenotypes and the location of mutations in relation to the NLS (Huppke and others 2002). The clinical phenotypes and type of *MECP2* mutations were analyzed in 123 patients with Rett syndrome at 5 years of age; four relationships between mutation type and location versus phenotype were identified. First, mutations affecting the NLS are more severe than those preserving the NLS. Second, deletions within the carboxy-terminus are less severe than other mutations. Third, truncations (except those occurring in the carboxy-terminus) are more severe than the missense mutations. Finally, missense mutations in the MBD are equivalent to missense mutations in the TRD (excluding those in the NLS). The authors of this study point out that some of the conflict in previous reports may be due to the admixture of carboxy-terminal deletions with the other truncating mutations. Cheadle and others (2000) had previously excluded the carboxy-terminal deletions as outliers and found an association between early truncations and increased severity. This result concurs with the findings of Huppke and others (2002) that truncations disrupting the NLS are more severe than those preserving the NLS.

Leonard and others (2003) further extended the genotype/phenotype analysis to the individual mutation level. Because R133C has relatively normal functioning based on in vitro models (vide infra), they proposed that patients with R133C may have a less severe phenotype. In their study, patients with R133C are less likely to be in the most severe category on a number of clinical measures—notably, speech, hand use, ambulation, and hand stereotypies. The overall clinical severity score is less severe in the R133C group, and there is no evidence of skewed XCI in the R133C group. These results sug-

gest that more information concerning genotype-phenotype relationships may be garnered by analysis of specific mutations rather than mutations grouped by type or location.

MECP2 *Mutations in PSVs*

The analysis of variants of Rett syndrome offers some insight into genotype-phenotype correlation. The PSV of Rett syndrome is a milder variant in which language functioning is relatively preserved compared with classic Rett syndrome (Zappella and others 2001). To date, 29 cases of PSV have been reported with mutations in *MECP2*. The mutations causing PSV have all also been reported in Rett syndrome, establishing that PSV and Rett syndrome share a molecular basis (Huppke and others 2000; Obata and others 2000; Hoffbuhr and others 2001; Nielsen and others 2001; Yamashita and others 2001; Zappella and others 2001; Conforti and others 2003; Weaving and others 2003). Although *MECP2* mutations in PSV are relatively equally distributed, with 38% in the MBD, 28% in the TRD, and 31% in the carboxy-terminus, the specific types of mutations are limited. In general, there is an overrepresentation of less severe mutations, such as R133C, carboxy-terminal deletions, and truncations after the NLS. A small number of cases have mutations affecting threonine 158 (T158), which is commonly mutated to methionine in classic Rett syndrome (T158M). It is interesting that two cases of PSV also have the T158M mutation but show a less severe phenotype than classic Rett syndrome. Both cases with PSV have skewed XCI (92:8) (Hoffbuhr and others 2001; Zappella and others 2001), which may indicate favorable skewing and thus allow a milder phenotypic expression. Two early truncations disrupting the NLS have been reported, but no information on XCI was obtained (Huppke and others 2000; Yamashita and others 2001). Thus, these phenotypically mild cases with otherwise severe mutations may be examples of the effects of skewed XCI patterns.

Phenotypes of Males with MECP2 *Mutations*

Rett syndrome has classically been described as only occurring in girls; however, case reports of boys with classic Rett syndrome have been reported (Jan and others 1999). Because familial cases of Rett syndrome suggested an X-linked gene with male lethality, it was generally assumed that few cases of Rett syndrome would exist in boys. After cloning the gene, a variety of boys has been identified with mutations in *MECP2* (Wan and others 1999; Villard and others 2000; Hoffbuhr and others 2001; Geerdink and others 2002; Zeev and others 2002). These boys fall into three categories: boys with Rett syndrome, boys with severe encephalopathy and infantile fatality, and boys with less severe neuropsychiatric phenotypes.

The boys with classic Rett syndrome carry the same mutations in *MECP2* as those that cause classic Rett syndrome in girls, but these boys have unique genetic features that rendered the Rett phenotype compatible with male viability. In three cases, the individuals are phenotypically male but have a 46, XXY karyotype associated with a condition called Kleinfelter's syndrome (Leonard and others 2001; Schwartzman and others 2001). An extra X chromosome in these males (and thus one normal copy of *MECP2*) reproduces the Rett phenotype. In three other cases, the boys appear to be somatic mosaics who have a mixed cellular population of wild-type and mutated *MECP2* (Clayton-Smith and others 2000; Armstrong and others 2001; Topcu and others 2002). This mixed cellular population is similar to the somatic mosaicism in girls, which occurs due to random XCI. One case has an unusual independent combination of a translocation of a portion of the Y chromosome (region of the SRY locus, which is sufficient to produce maleness) onto a paternal X chromosome with a *MECP2* mutation. Thus, this phenotypic male has a typical female karyotype (46, XX) and classic Rett syndrome (Maiwald and others 2002).

One interesting case of a boy with classic Rett syndrome has a mutation in *MECP2* but no evidence of either somatic mosaicism or Kleinfelter's syndrome (Ravn and others 2003). The mutation is unique (817dup7) but within the TRD and predicted to be similar to others described in girls with Rett syndrome. Mutations similar to this are found to cause severe neonatal encephalopathy in boys (vide infra): The differences in the phenotypes among males carrying this mutation suggest additional genetic factors modifying the phenotypic spectrum in boys.

In some familial cases of Rett syndrome, male siblings were born with severe encephalopathy and died by 1 to 2 years of age. Genetic analysis revealed that these boys contained identical *MECP2* mutations to those seen in their sisters with Rett syndrome (Wan and others 1999; Villard and others 2000; Hoffbuhr and others 2001; Geerdink and others 2002; Zeev and others 2002). This indicates that Rett syndrome is a milder manifestation of these particular mutations in *MECP2*, and boys with a complete absence of wild-type *MECP2* develop more severe phenotypes. The majority of *MECP2* mutations that cause Rett syndrome in girls and the severe encephalopathy in boys either are in the MBD or disrupt the NLS, either of which is predicted to completely disrupt the function of MeCP2.

Recently, boys with less severe, non-Rett phenotypes and mutations in *MECP2* have been discovered. These boys were identified in two ways. The first cohort was discovered by examining families with segregation of X-linked mental retardation (Meloni and others 2000; Couvert and others 2001; Dotti and others 2002; Klauck and others 2002; Kleefstra and others 2002; Winnepenninckx and others 2002; Yntema, Oudakker, and others 2002), and the second cohort was found by analyzing groups of unrelated boys identified because they have mental retardation, features of Angelman syn-

drome, or neuropsychiatric conditions such as psychosis (Couvert and others 2001; Imessaoudene and others 2001; Cohen and others 2002). The phenotypes in these boys are broad with few unifying features. One common feature is some degree of mental retardation, primarily in the moderate range. Additional features include tremor, hypotonia, mood instability/psychosis/mania, obesity, and gynecomastia. The boys share a common molecular genetic theme: The types of *MECP2* mutations in these boys are never identified in girls with Rett syndrome, presumably because their effects are mild when in heterozygosity. Indeed, the obligate female carriers in familial X-linked mental retardation kindreds have mild mental retardation or are reportedly phenotypically normal (Meloni and others 2000; Orrico and others 2000; Klauck and others 2002).

Couvert and others (2001) have suggested that *MECP2* mutations are a relatively common (1%–2%) cause of mental retardation in boys; however, others argue that many of these identified mutations may actually be benign polymorphisms that do not actually contribute to the phenotype (Laccone and others 2002; Yntema, Kleefstra, and others 2002). In fact, a study from Holland found *MECP2* mutations in only 0.2% of mentally retarded boys (Yntema, Kleefstra, and others 2002). These groups argue for pedigree analysis and extensive sequencing of *MECP2* in phenotypically normal males to separate causative mutations from polymorphisms.

Milder phenotypes in boys generally involve the C terminus of MeCP2, with the exception of three missense mutations (A140V, E137G, and R167W) within the MBD. As will be discussed below, A140V has interesting properties in vitro. Although A140V is the most frequently identified mutation in males (found in 17 cases), this mutation has not been reported in classic Rett syndrome. This is interesting because A140V is the result of a C-T transition at a CpG hotspot: Similar C-T transitions cause the eight most commonly seen mutations found in classic Rett syndrome (Lee and others 2001). In addition, a late truncation (R406X), which is also the result of a C-T transition, is identified in males with mental retardation and progressive spasticity but not found in classic Rett syndrome (Meloni and others 2000). The fact that two mutations resulting from C-T transitions are common in boys with *MECP2* mutations but are not found in girls with Rett syndrome suggests that these mutations uniquely alter MeCP2 function in a manner milder than mutations causing Rett syndrome.

In Vitro Analysis of Some *MECP2* Mutations

In a thorough in vitro analysis of known mutations in the MBD of MeCP2, Kudo and others (2003) found a correlation between the ability to bind the heterochromatic regions and to repress methylated promoters. Interestingly, they found differential effects on function between the residues that are predicted to contact the pair of methylated cytosines. Specifically, mutations affecting R111 completely abolish ability to bind heterochromatic regions and do not repress transcription from a methylated promoter, whereas mutations affecting the residues contacting the other methyl group, R133C and R134C, have no effect on the focal nuclear staining and no noticeable effect on the ability to repress transcription from a methylated promoter. The disparity is also seen at the level of the residues that contact the sugar backbones and deoxyribose moieties. No mutations are seen in the residues that contact the backbone of the methyl group contacted by R111. Many of the mutations in the residues that interact with the complementary DNA strand (K135E, E137G, A140V, T158A, and T158M) do not cause a strong effect on MeCP2 function in this in vitro assay. In fact, A140V performs nearly identical to wild type.

The A140V mutation is interesting in a number of additional ways. It seems to interact promiscuously with unmethylated promoters (Kudo and others 2003), similar to mutations in R133 and R134. These results combine to suggest that R111 provides the critical contact of a methyl group, whereas the residues interacting with the complementary strand provide the specificity for both methylated DNA and the context of specific DNA sequences. It is especially interesting that the missense mutations commonly found in boys with non-Rett phenotypes cluster to residues on this complementary strand. Furthermore, as outlined previously, R133C appears to be less severe clinically in girls with Rett syndrome. Extending the analysis of these mutations into in vivo systems will provide additional insight.

MeCP2 Expression during Development

Interesting work demonstrates dynamic MeCP2 protein levels in the developing nervous system. Although the mRNA expression is relatively uniform in the brain and other tissues of the mouse embryo, examination of protein level reveals extensive posttranscriptional control of MeCP2 abundance. Dynamic modulation of levels of MeCP2 protein within the nervous system mirrors neuronal maturation. In the mouse, the protein is seen initially within the spinal cord, lower brain stem, and the Cajal-Retzius neurons of the marginal zone of the cortex (Shahbazian, Antalffy, and others 2002). As development progresses, additional regions reveal detectable levels of MeCP2, such as the cerebellum, deep cortical neurons, thalamus, and caudate. Finally, the hypothalamus, hippocampus, and deep cerebellar nuclei become MeCP2 positive. At birth, all neurons in the cortex stain for MeCP2, but the deeper neurons stain more robustly than the superficial neurons, reflecting the earlier maturation of deeper neurons. Postnatally, greater numbers of neurons in the cortex become MeCP2 positive. These results demonstrate that the timing of appearance of MeCP2 correlates with the ontogeny of the CNS and continues to increase with maturation. Similar developmental control of the expression of MeCP2 protein is seen in another rodent, the rat (Jung and others 2003).

The level of MeCP2 protein has been analyzed in two primates, macaque monkeys (Akbarian and others 2001) and humans (Shahbazian, Antalffy, and others 2002; Balmer and others 2003). The overall pattern of expression is generally the same as seen in rodents: As neurons mature, they express MeCP2. An interesting finding in humans is the increase in the number of cortical neurons that become MeCP2 positive during childhood. Comparing 3-month-old brains with 10-year-old brains reveals an observable increase in the percentage of MeCP2-staining cortical neurons. This is in contrast to the deeper structures in the human brain (such as the reticular formation) in which a constant percentage of MeCP2-positive neurons are present from 35 weeks. In the subhuman primate studied, the cortical neuronal MeCP2 protein levels reach adult levels in the perinatal period, just like in the rodents studied.

In Vivo Studies of MeCP2 and Other MBDs

Mouse Models of Rett Syndrome

Three mouse models of Rett syndrome have been generated using embryonic stem cell gene replacement technology. The three models differ in the type of mutations generated, the severity of the mutant phenotypes, and the exact tissues in which MeCP2 function is altered.

Cre-Lox technology was used to generate the first two mouse models. This technology allows the removal of specific DNA sequences flanked by Lox sequences (flox-ed) by Cre mediated recombination. Cre recombinase expression may be controlled by fusing the DNA sequence coding for Cre to a known promoter. Guy and others (2001) used a ubiquitous promoter (deleter) to generate early embryonic elimination of *Mecp2*. These null mice are initially normal but develop tremors between 3 and 8 weeks of age. In addition, breathing irregularities and jaw misalignment are observed. Gross and histological analysis of the brains reveals no abnormalities. The mice have a shortened life span, dying at approximately 54 days.

The phenotype of the null mice generated by Chen and others (2001) was similar to that seen by Guy and others. The mice are initially normal but develop tremors at approximately 5 weeks of age. Likewise, no gross abnormalities in brain architecture are found, except smaller and denser neurons, similar to girls affected by Rett syndrome. These mice also have a shortened life span, dying by 10 weeks.

To explore the role of *Mecp2* solely in neuronal lineages, both groups used Nestin promoter fused to Cre to selectively remove *Mecp2* from these lineages (Chen and others 2001; Guy and others 2001). Mice lacking *Mecp2* in neurons are phenotypically identical to mice lacking *Mecp2* in all tissues, demonstrating that removal of *Mecp2* from neurons is sufficient to re-create the phenotype. This result is not inherently expected, as *Mecp2* is expressed in a variety of non-CNS tissues. Extending the

analysis, Chen and others (2001) used a Cam kinase promoter to eliminate *Mecp2* expression from postmitotic neurons in the forebrain, hippocampus, and brainstem. These mice display a phenotype similar to mice completely lacking *Mecp2*, except the onset of symptoms is delayed until approximately 3 months. This demonstrates that *Mecp2* is not required for proper development of neurons but rather required to maintain proper postmitotic function of neurons.

Shahbazian, Young, and others (2002) reasoned that the phenotypes seen in the null male mice were more severe than those seen in human girls with Rett syndrome and were more similar to the severe disease seen in boys with Rett-causing mutations. Therefore, to create a milder mutation, they generated a mouse with truncated *Mecp2* by engineering a stop codon after codon 308. A truncated protein is created that contains the MBD, TRD, and NLS but removes the last third of the protein. This mutation was designed to be situated between a truncating mutation that causes severe encephalopathy and death in boys and a milder mutation that allows male survival with severe retardation and ataxia. The phenotype of the male mice with this truncation recapitulates many of the features seen in girls with Rett syndrome. The mice are normal until around 6 weeks of age, at which time they develop a slight tremor. This tremor worsens over time, and the mice develop kyphosis after 5 months of age. Some mice develop seizures and myoclonic jerks. The mice perform less well than wild-type mice on motor tasks but show no learning abnormalities in a conditioned fear task or spatial learning task. Unexpectedly, the mice develop repetitive forepaw movements similar to the hand wringing seen in Rett syndrome. The mice also show anxiety, decreased grooming, and abnormal social interactions with wild-type mice. There are no obvious structural abnormalities of the brain, including no alteration in the expression of molecular markers such as glial fibrillary acidic protein or microtubule-associated protein 2. There is, however, hyperacetylation of histone H3 in the mutant mice. The majority of these mice survive up to 1 year.

Other MBD Mutant Mice

Mice lacking other methyl-binding domain proteins have also been generated. *Mbd3* is required for proper embryogenesis, as mice lacking *Mbd3* do not survive past 8.5 days postconception (Hendrich and others 2001). *Mbd2*-null mice are viable and fertile and show no structural abnormalities (Hendrich and others 2001). In fact, these mice only manifest nurturing behavior abnormalities in the null females. The pups raised by *Mbd2*-null female mice are underweight and less likely to survive than those raised by wild-type mothers. This effect is seen even when wild-type mice are raised by *Mbd2*-null mothers, demonstrating that the genotype of the mother, not the pups, determines the nurturing phenotype. The removal of a single copy of *Mbd3* in mice lacking *Mbd2* reduces viability, demonstrating a genetic

interaction between these two proteins (Hendrich and others 2001).

Mbd1 knockout mice have recently been generated and are viable and fertile (Zhao and others 2003). In contrast to *Mecp2* mutant mice, they display no motor or coordination abnormalities but rather have decreased spatial learning and reduction in long-term potentiation in the dentate gyrus of the hippocampus. Furthermore, the adult neuronal stem cells in these mice have reduced ability to generate new neurons and increased genomic instability. *Mbd1* expression is predominately in the hippocampus; thus, the results concerning reduced spatial learning and long-term potentiation are not unexpected.

One possibility is that *Mbd1* and *Mecp2* may have overlapping functions in certain neurons but distinct roles in other neurons. A possible model might be that *Mecp2* is required for the maintenance of correct motor function and social behavior, whereas *Mbd1* is required for the proper functioning of the hippocampus and thus spatial learning. Given the genetic interactions found between *Mbd2* and *Mbd3*, double mutation analysis between *Mecp2* and *Mbd1* is needed to determine if any genetic interactions exist.

Role of MeCP2 in Xenopus Development

The role of MeCP2 in early Xenopus development has recently been analyzed by Stancheva and others (2003). Xenopus MeCP2 (xMeCP2) expression is restricted to the developing nervous system after the midblastula transition, and later expression is high in all neural tissue. Proper development requires xMeCP2: Inhibition of xMeCP2 expression by antisense injection results in alteration of the dorsal axis and head structures. Coinjection of human MeCP2 mRNA rescues this effect. Near-complete rescue is obtained when a known late missense mutation (R306C) is coinjected but only partial rescue when an early MeCP2 truncating mutation (R168X) is coinjected.

Although injection of antisense MeCP2 or the R168X mutant RNA does not alter the expression of proneural genes, expression of genes downstream of the proneural genes is altered. The two RNA constructs have opposing effects on the cell fate determination. Specifically, removal of MeCP2 by antisense injection results in a loss of neuronal cells, as indicated by increased expression of a nonneuronal marker, *Hairy2a* (Fig. 2B). Expression of mutant MeCP2 (R168X) results in excess neuronal cells and decreased expression of *Hairy2a* (see Fig. 2C). Whole mount in situ mRNA hybridization confirms this: Injection of antisense MeCP2 results in loss of differentiated primary neurons, and injection of R168X results in expansion of lateral neurons.

By focusing on the promoter of *Hairy2a*, a specific downstream target of the Notch/Delta pathway, Stancheva and others (2003) dissected the molecular interactions of MeCP2 at the DNA level. MeCP2 interacts with methylated DNA in the *Hairy2a* promoter to regulate *Hairy2a* expression. When bound to methylated DNA, MeCP2 interacts with the SMRT corepressor complex indirectly via interaction with Sin3A through MeCP2 TRD (Fig. 2). Notch signaling removes SMRT from the promoter, and removal of SMRT causes MeCP2 to fall off DNA (Fig. 2A). Interestingly, this interaction does not occur when the R168X mutation is expressed. Rather, R168X continues to bind to methylated DNA (Fig. 2C). Thus, R168X acts differently from complete absence of MeCP2 (Fig. 2B), in terms of both cell fate determination and molecular interactions. It appears that R168X may function as a dominant negative molecule. This has ramifications in respect to the mouse models thus engineered (Chen and others 2001; Guy and others 2001; Shahbazian, Young, and others 2002). Two are complete nulls and may function in a manner different from known Rett mutations. Also interesting is that in Xenopus, xMeCP2 is involved in neurogenesis, similar to the role proposed for mouse *Mbd1* (Zhao and others 2003), whereas mouse *Mecp2* does not appear to affect neurogenesis. This difference may reflect a difference in primary neurogenesis in Xenopus or additional redundancy of methyl-CpG-binding proteins in the mouse.

MeCP2 in Developing and Regenerating Olfactory System of Rodents and Humans

The olfactory system is suitable for the analysis of MeCP2 function in neuronal development. It is easily obtained via the olfactory epithelium in the nose and is well characterized, and development occurs along a defined trajectory (Graziadei and Graziadei 1979). Furthermore, specific manipulations induce regeneration of the epithelium in a defined manner.

Cohen and others (2003) made use of the olfactory system to monitor the expression of MeCP2 during development and regeneration. In agreement with previous MeCP2 studies, MeCP2 levels increase in more mature neurons in both normal development and regeneration. The relation of MeCP2 abundance to synapse formation is important to help elucidate the role of MeCP2 in neuronal maturation. A set of molecular markers that distinguish nonsynapsed neurons from mature neurons allows the determination that MeCP2 is found in the nonsynapsed neurons. In an experimental model that prevents synapse formation, MeCP2 is also present, albeit at a lower level than normal. Thus, the presence of MeCP2 does not require synapse formation but protein level increases when synapses form. This suggests that mature neurons that have formed synapses induce full MeCP2 levels, and this level may be critical for maintaining or modulating synapses. Further experiments in which the olfactory neuronal development is monitored in the absence of MeCP2 are needed to clarify the role of MeCP2 in maturation.

Using the olfactory epithelium in humans as a model, Ronnett and others (2003) analyzed olfactory biopsies from girls with Rett syndrome versus normal controls. Although the overall number of neurons is not obvious-

ly different in girls with Rett syndrome, an excess of immature neurons in Rett syndrome is found. The mature neurons present in Rett syndrome are abnormally structured, with absent or unusual dendrites, dispersed axons, and dysmorphic, small cell bodies. These results indicate that MeCP2 is required for the formation or maintenance of mature neurons and dendrites in the olfactory epithelium and support the belief that MeCP2 is required for proper neuronal maturation.

Pathogenesis of Rett Syndrome and Other Related Neurodevelopmental Disorders

Certain clinical features suggest a similarity between Rett syndrome and other neurodevelopmental disorders, most notably autism. During certain phases of the disease, girls with Rett syndrome display autistic features. Furthermore, autism and Rett syndrome share an initial period of normal development. Both conditions also have alteration in social interaction, language, and stereotypies. Do autism and Rett syndrome share any other similar pathological features?

The gross structure of the brain is preserved in both Rett syndrome and autism (Armstrong 2002; Kemper and Bauman 2002), although the brains in autism are slightly larger than normal brains (Herbert and others 2003) in contrast to the smaller than normal brains found in Rett syndrome (Armstrong 2002). Microscopically, the brains in Rett syndrome and autism share certain similarities. In either condition, there is no abnormality in myelin, no neuronal migration defects, and no consistent evidence of cell loss or atrophy (Armstrong 2002; Kemper and Bauman 2002). There is, however, a decrease in the size of neurons and increased neuronal packing in both Rett syndrome and autism. Furthermore, Golgi analysis revealed a reduction in the dendritic arborization in both Rett syndrome and autism, albeit in different brain regions (Armstrong and others 1995; Bailey and others 1995; Raymond and others 1996; Kemper and Bauman 1998). Dendritic arborization is related to synaptic input: In Rett syndrome, the density of synapses is decreased (Belichenko and others 1994). Together, the data indicate that one of the primary alterations in the brains of girls with Rett syndrome and children with autism is a decrease in dendrites and synapses in selective areas of the brain. This begs the question, Is there any genetic or molecular data that connect the two conditions?

Autism has long been suspected of having a genetic contribution, most evident by the higher concordance rate in monozygotic twins (70%–90%) compared with that in dizygotic twins (0%–10%) (Bailey and others 1995). The most commonly reported chromosomal alteration in autism is extra copies of 15q11-q13. As this region is also involved in Angelman syndrome, it is interesting that MeCP2 mutations have also been described in children with the clinical features of Angelman syndrome (Imessaoudene and others 2001). The possible connection between Rett syndrome and autism has led to genetic analysis of children with autism to determine if they have mutations in *MECP2*. Despite previous reports that have not found any mutations (Vourc'h and others 2001; Beyer and others 2002; Lobo-Menendez and others 2003; Zappella and others 2003), *MECP2* mutations have recently been discovered in patients with autism and no other features of Rett syndrome (Carney and others 2003). Recent genetic data have also demonstrated mutations in neuroligin-3 (NLGN-3) and NLGN-4 in two cases of familial autism (Jamain and others 2003).

Neuroligins are a family of postsynaptic proteins that were originally discovered due to their ability to form molecular complexes with β-neurexins (Ichtchenko and others 1995). Neuroligins are transmembrane proteins that interact intracellularly with postsynaptic proteins (Irie and others 1997; Kurschner and others 1998). In fact, NLGN-1 localizes to the postsynaptic membrane and clusters NMDA-1 receptors (Song and others 1999). Neurexins are a family of three genes expressed in the brain that also code for transmembrane proteins. Intracellularly, neurexins interact with a presynaptic protein, Cask, which associates with proteins that affect vesicle transport (Butz and others 1998).

The physical interaction of neurexins and neuroligins and their respective interaction with presynaptic and postsynaptic proteins suggest a possible role in the creation of asymmetry at the synapse. Previous cell adhesion molecules such as cadherins that localized to the synapse displayed homotypic interactions, thus precluding them from creating asymmetry. Nguyen and Sudhof (1997) demonstrated that cells expressing neurexin-1β form aggregates with NLGN-1-expressing cells, revealing their ability to function as heterotypic cell adhesion molecules. In an exciting extension of these experiments, Scheiffele and others (2000) showed that expression of NLGN-1 or -2 in non-neuronal cells (HEK293) induces presynaptic differentiation in primary cultures of pontine neurons with NLGN-1- or NLGN-2-expressing non-neuronal cells. These synapses were both structurally intact and functional. These data have yet to be reproduced with those neuroligins implicated in familial autism (NLGN-3, -4) but strongly suggest that the genetic alteration in these familial autism cases involves a biochemical alteration of the synapse.

Thus, neuropathological data point to an alteration of dendrites and synapses in Rett syndrome and autism, and genetic data point to alterations of the synapse in autism. Although further genetic work is needed to demonstrate mutations in NLGN-3 and -4 in sporadic autism, this does allow molecular insight into the pathogenesis of autism. More extensive studies in Rett syndrome into the details of synapse formation and the expression of genes such as NLGN are needed to explore the relationship.

A variety of other neurodevelopmental syndromes, such as Angelman syndrome, Fragile X syndrome, tuberous sclerosis, Down syndrome, untreated phenylketonuria, and mental retardation with infantile spasms,
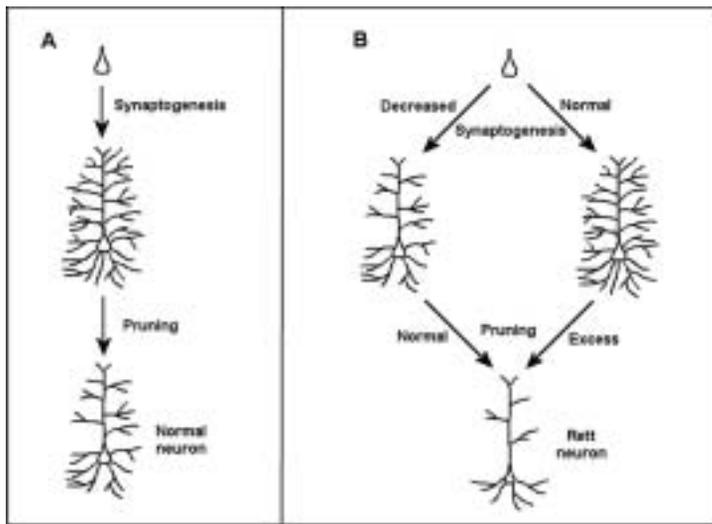
Fig. 3. Synaptogenesis and pruning. During the normal course of neuronal maturation, initially exuberant synaptogenesis results in excess synapse formation (see *A*). During the course of maturation, synapse numbers are reduced through a process termed *pruning*. Pruning occurs in different cortical regions at different times during development and reflects the differential timing of neuronal maturation in different cortical regions. In neurodevelopmental conditions such as Rett syndrome, a smaller dendritic arbor and less dendritic spines are seen, which likely reflects decreased synapses (see bottom of *B*). Two possible routes to this final outcome are diagrammed in *B*. The left side of the figure represents decreased initial synaptogenesis with normal pruning, ultimately resulting in decreased number of synapses. On the right side of *B*, diagrammed is a situation in which normal initial synapse proliferation is followed by excessive pruning. The final state of neurons in Rett syndrome may be achieved by either pathway or a combination of these pathways.

has been found to have alterations in dendrites and synapses (Huttenlocher 1974, 1991, 2000; Jay and others 1991; Irwin and others 2000). Are these neurodevelopmental syndromes ultimately dendritopathies/synapsopathies? An interesting question is the exact sequence of events. Seminal work by Huttenlocher demonstrated a temporal sequence of synaptogenesis in humans, which varied by cortical location (Huttenlocher 1979; Huttenlocher and others 1982). Synapses increase rapidly in early life and then are pruned to an adult level (Fig. 3A). Is the paucity of dendritic arbors seen in Rett syndrome, autism, and other neurodevelopmental disorders due to impairment in synaptogenesis, or rather, does it result from excess in pruning (Fig. 3B)? The data in Down syndrome suggest that there is initially an increase in arborization followed by excess pruning. Experimental models of phenylketonuria and hypothyroidism also demonstrate increased dendritic spines initially followed by excessive pruning (see references in Huttenlocher 1991). Although these three conditions have an overabundance of pruning, the link between autism and the synaptogenic molecules such as neuroligins suggests a situation in which impaired synaptogenesis dominates. We stand at the point in which these questions may be posed, and the use of genetic, biochemical, and neurobiological techniques on well-defined model systems such as those available for Rett syndrome may allow insight into these fundamental issues of neuronal development and function.

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