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Methylated Cytosine and the Brain: A New Base for Neuroscience

Minireview

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Methylation of genomic CpG residues is crucial for proper neuronal function. Rett syndrome, a common form of mental retardation, is associated with mutations in the gene encoding MeCP2, a methyl CpG binding protein linked to transcriptional repression. Gene knockouts of mouse Mecp2 have reproduced key aspects of the disease. A CNS-restricted knockout of Dnmt1, encoding the enzyme that maintains CpG methylation patterns, results in loss of mutant neurons and glia.

In the genome of many eukaryotes, cytosine residues are covalently modified by the addition of a methyl group preferentially, but not exclusively, at the sequence CpG. In mammals, 60%–90% of CpG sites are methylated, and most of the remaining unmethylated residues are clustered in CpG islands within functional gene promoters. Changes in methylation status can result in profound changes in gene expression, mostly in which methylation correlates with an inactive transcriptional status. Methylated CpG residues are specifically recognized by a small family of transcriptional repressors, which upon binding can recruit members of chromatin remodeling complexes, leading to transcriptional inactivation after the deacetylation of core histone proteins (reviewed in Robertson and Wolffe, 2000). In mouse and *Xenopus*, disruption of normal genomic methylation patterns radically disturbs development after gastrulation, leading to apoptosis throughout the embryo (Stancheva et al., 2001, and references therein). Although years of in vitro experiments suggested a role for DNA methylation in the tissue-specific regulation of gene expression, the embryonic lethality seen in the aforementioned experiments prevented an investigation of its relevance in vivo. Bringing together complementary genetic approaches in human and the mouse, reports in the past 2 years have revealed that methylated cytosine represents a new base for neuroscience in the study of neuronal stability, morphology, and function.

As in all other parts of the body, mammalian genomic DNA in the brain is methylated throughout life, and brain extracts contain methyltransferase activity. The first indication that DNA methylation could be important for the central nervous system (CNS) was unexpectedly provided by the protein MeCP2. MeCP2 binds to individual methylated CpG residues, and it has been shown to recruit members of chromatin remodeling complexes, such as the transcriptional repressor Sin3A and the histone deacetylases HDAC1 and HDAC2 to effect the silencing of genes (Figure 1; Jones et al., 1998; Nan et al., 1998). Dozens of mutations have been identified in the *MECP2* gene in patients with Rett Syndrome, one of the most common genetic causes of mental retardation in females. Patients appear normal for the first 6–18 months of life, then rapidly degenerate, suffering from severe dementia and autistic behavior (references in Chen et al., 2001). The disease follows a slow progression involving truncal ataxia, loss of intentional hand use, stereotypical hand movements, and a high incidence of epilepsy. Examination of the brain revealed no evidence of tissue degeneration, but a pronounced microcephaly, caused at least in part by a decrease in neuronal cell size and an increase in packing density. MeCP2 is an X-linked gene that undergoes X chromosome inactivation, so heterozygous mutant females are functionally mosaic for the mutation. However, this mosaicism is not evident in postmortem examination of brain tissue, which revealed a uniform reduction in the length and complexity of dendritic branching in neurons of layers III and V of frontal and motor cerebral cortex (Armstrong et al., 1998). Such reductions were seen in all examined neurons, suggesting a non-cell-autonomous effect of the mutation. Rett mutations comprise two varieties: missense mutations occurring mostly in the amino-terminal methyl CpG binding domain (MBD), and nonsense mutations, which are predicted to cause protein truncations mostly 3′ to the MBD in the transcriptional repression domain (TRD; reviewed in Van den Veyver and Zoghbi, 2000). Recombinant MeCP2 proteins bearing missense mutations in the MBD have been shown in vitro to lose the ability to bind methylated DNA (Yusufzai and Wolffe, 2000). Recently, Wan et al. (2001) examined fibroblasts from Rett patients bearing two of the more common truncation mutations and showed that the acetylation of histone H4 was increased, as would be expected for mutants lacking the TRD.

MeCP2 is ubiquitously expressed in human and mouse; are the neurological defects seen in Rett patients caused exclusively by defects in the CNS? Also, is the delay of onset of the disease indicative of a destabilization in postnatal neuronal function or the delayed appearance of a developmental defect? The laboratories of Bird and Jaenisch (Guy et al., 2001; Chen et al., 2001) present mouse models of Rett syndrome that answer both of these questions. In both studies, protein-coding exons of the *Mecp2* gene were engineered with flanking bacterial loxP sites, so that *Mecp2* could be eliminated through recombination after expression of Cre recombinase. Ubiquitous deletion of *Mecp2* resulted in mice that appeared normal until several weeks of age, when they developed neurological abnormalities such as abnormal gait and reduced activity. As with Rett syndrome patients, the mice display microcephaly and smaller neuronal size (Chen et al., 2001); however, all homozygous mutants died within several months. Amazingly, heterozygous mutant females, as in humans, developed inertia and ataxic-like symptoms 6 months after birth, despite the huge difference in developmental time scales between the two species. By crossing to a strain of mice

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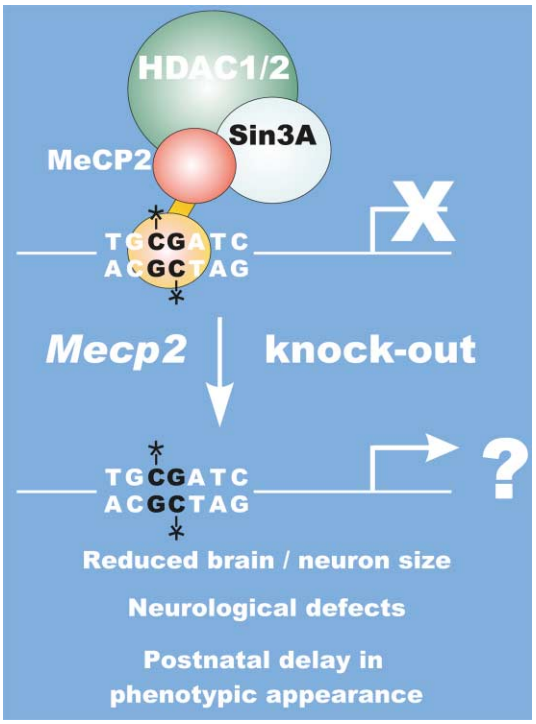

Figure 1. Murine *Mecp2* Knockout Reproduces Aspects of Rett Syndrome

MeCP2 specifically binds methylated CpG residues (asterisks indicate methyl groups). The transcriptional repression domain of MeCP2 recruits Sin3A and histone deacetyltransferases (HDAC1/2) to affect transcriptional repression (X) of an unidentified group of target genes. In the *Mecp2* knockout, Rett syndrome-like phenotypes are possibly caused by aberrant transcription (?) of these target genes.

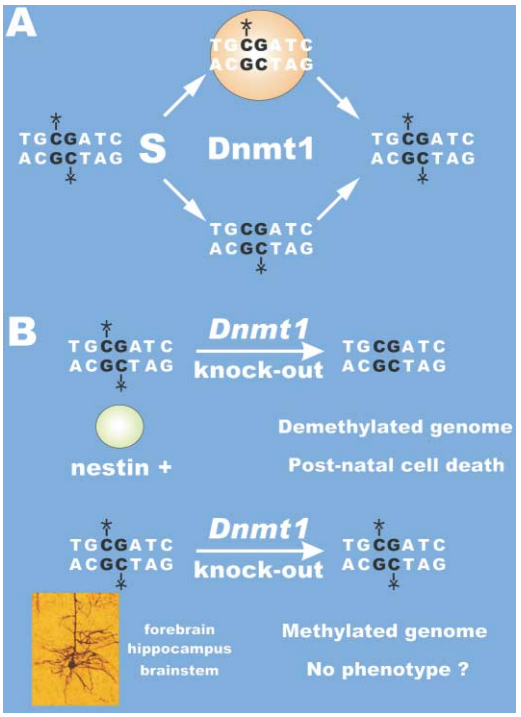

Figure 2. A Requirement for Genomic Methylation in the Central Nervous System

(A) Cytosine is preferentially methylated (asterisk) at CpG residues in genomic DNA, and this methylation pattern is faithfully reproduced during DNA replication (S) by the action of DNA methyltransferase 1 (Dnmt1, orange circle), which shows a preference for hemimethylated DNA.

(B) Dnmt1 is highly expressed in neurons, a postmitotic population. A loss of *Dnmt1* in proliferating neuronal precursors (nestin +) generates highly demethylated neurons and glia that are rapidly eliminated postnatally. In contrast, loss of *Dnmt1* in neurons in the forebrain, hippocampus, and brainstem leads to no demethylation and no apparent phenotype.

expressing Cre recombinase from the nestin promoter/enhancer, both laboratories were able to produce mutants in which almost all CNS neurons and glia were missing *Mecp2*. These mice developed identical symptoms in the same time frame as the complete mutants.

The Jaenisch laboratory used, in addition, a mouse expressing Cre recombinase from the Cam kinase promoter, to affect a knockout of *Mecp2* throughout the brain but exclusively in postmitotic neurons. In these animals, similar neurological phenotypes were observed, including an ataxic gait, reduced activity, and a decrease in brain weight and neuronal size. Although these phenotypes developed at later time points, this restricted knockout clearly demonstrates that the Rett-like phenotypes originate exclusively from a neuronal deficit of *Mecp2*, which leads to a disturbance in their postnatal function. The correlate question is of course then raised: why should a ubiquitous loss of *Mecp2* exhibit a phenotype primarily in the CNS? The specificity of the *Mecp2* knockout phenotype could reflect a lack of complementation by other methyl-CpG binding proteins in the brain. *Mbd1*, *2*, and *3* are three other members of the Mecp2 family (references in Hendrich et al., 2001), and Bird's group addressed the possibility of functional redundancy by crossing the *Mecp2* knockout mouse to a strain lacking *Mbd2*. *Mbd2* acts as a transcriptional repressor in vitro and copurifies with the MeCP1 histone deacetylase complex, another chromatin-remodeling complex implicated in transcriptional repression (Ng et al., 1999). Interestingly, the *Mbd2* knockout also showed a behavioral phenotype, in that mutant mothers did not adequately feed their young (Hendrich et al., 2001). With respect to the Rett-like phenotype, double mutant animals showed identical symptoms and mortality as the *Mecp2* knockout alone, and no new obvious defects. *Mbd1*, *2*, and *3* are expressed throughout the body, but any full explanation of possible functional redundancy must be predicated on the basis of spatiotemporal expressional profiles, which are notably lacking for these genes.

Rett syndrome could also reflect a particular sensitivity of neurons to methylation-dependent transcriptional repression. A separate line of evidence suggests that genomic methylation must be tightly controlled for neurons to survive. CNS neurons demonstrate extremely high levels of expression of *Dnmt1* (Inano et al., 2000), which encode a maintenance methyltransferase showing a preference for the hemimethylated DNA substrates seen directly after replication of genomic DNA (Figure 2A). *Dnmt1* activity is known to be spatially and temporally coupled to DNA replication sites in cycling cells, so

it is puzzling why it should be expressed in a postmitotic cellular population that has inherited a methylated genome from cycling precursors. Even more puzzling, most of the Dnmt1 protein is localized to the neuronal cytosol (Inano et al., 2000). Fan et al. (2001) utilized the identical cre-loxP system described above to inactivate *Dnmt1* specifically in the CNS (Figure 2B). Mice showing a nearly complete loss of *Dnmt1* in the CNS died shortly after birth because of an unexplained respiratory failure. Although gross genomic hypomethylation could be detected as early as 12.5 days postcoitum, an examination of the brain in newborns revealed no obvious developmental abnormalities. However, and most curiously, mice whose brains bore a low fraction of *Dnmt1*-negative cells showed a rapid loss of these cells, leading to complete elimination of mutant neurons and glia 3 weeks after birth. Although it is totally unclear how gene-specific methylation patterns are altered in the neurons of these mice, clearly methylation is needed for survival of cells in the CNS, in a manner which seems to be cell autonomous.

A second knockout restricted to postmitotic neurons showed neither genomic hypomethylation nor any obvious neuronal loss. Though this may seem unsurprising given that normal methylation patterns were in place when the maintenance methyltransferase was removed, there remains the question why neurons retain so much Dnmt1 protein. Under certain conditions, Dnmt1 possesses de novo methylase activity. It should be stressed that the two other functional methyltransferase genes, *Dnmt3a* and *3b*, are known to encode de novo methylation activities and are also expressed, although at lower levels, in the brain. Although there is no evidence for an involvement of methylation in plastic CNS processes, such as synaptic or dendritic remodeling, transcriptional changes and protein expression are known to be crucial for the establishment of many forms of long-term memory (reviewed in Davis and Squire, 1984). It is conceivable that de novo DNA methylation could affect these processes, either through its effect on global repression of gene activity, or its specific modification of genes involved in such processes. A more plausible activity of *Dnmt1* in neurons would be to remethylate cytosine residues that have undergone DNA mismatch repair, which would occur after the spontaneous deamination of methyl cytosine to thymine residues (Brooks et al., 1996). Maintenance of genetic and epigenetic integrity is expected to be critically important for those cells, like neurons, that have to function for the lifetime of the animal. To investigate these possibilities will require examination of potentially subtle defects in the *Dnmt1* mutant mice.

Taken together, these studies clearly indicate that methylated cytosine and the proteins that bind it are essential for both proper CNS survival and function. Although drastically hypomethylated, glia and neurons can form but are quickly eliminated from the postnatal brain. In the more conservative case of the removal of MeCP2, one of the interpreters of the methylation mark, the entire brain shrinks in size and proper neuronal morphology and function is severely compromised in certain areas. So what is the importance of DNA methylation for neurons? The absence of methylation in *Xenopus* and mice clearly results in massive developmental arrest, which is preceded by the disregulation of stage-specific genes. Aside from its known specific effects on the monoallelic expression of imprinted genes, methylation may serve to decrease general transcriptional "noise." Why neurons should be particularly sensitive to aberrant transcription of this order is unclear. Studies are now appearing that look at the effects of hypomethylation upon global gene expression. Using oligonucleotide microarray analysis, fibroblast cultures deficient in *Dnmt1* were shown to deregulate expression of up to 10% of the examined genes (Jackson-Grusby et al., 2001). Similar studies comparing the misexpression of genes in the CNS-specific *Dnmt1* and *Mecp2* knockouts are doubtless underway.

Neither the Bird nor the Jaenisch laboratory reported the rapid postnatal loss of neurons from the *Mecp2* knockout animals, suggesting that gross genomic hypomethylation, as seen in the *Dnmt1* knockout, may result in the misexpression of a larger subset of genes necessary for neuronal survival. Another possibility is a loss of chromosomal stability, as seen in ICF syndrome, a disease recently shown to be caused by mutations in the *DNMT3B* gene (references in Robertson and Wolffe, 2000). Embryonic stem cells deficient in *Dnmt1* also show genomic instability, as seen in increased mutation rates and somatic recombination (Chen et al., 1998). The structural stability that CpG methylation brings to chromosomes may prove to be equally important in explaining the decreased viability and stunted morphology of neurons deficient in this process.

Selected Reading

Armstrong, D.D., Dunn, K., and Antalffy, B. (1998). J. Neuropathol. Exp. Neurol. *57*, 1013–1017.

Brooks, P.J., Marietta, C., and Goldman, D. (1996). J. Neurosci. *16*, 939–945.

Chen, R.Z., Pettersson, U., Beard, C., Jackson-Grusby, L., and Jaenisch, R. (1998). Nature *395*, 89–93.

Chen, R.Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Nat. Genet. *27*, 327–331.

Davis, H.P., and Squire, L.R. (1984). Psychol. Bull. *96*, 518–559.

Fan, G., Beard, C., Chen, R.Z., Csankovszki, G., Sun, Y., Siniaia, M., Biniszkiewicz, D., Bates, B., Lee, P.P., Kuhn, R., et al. (2001). J. Neurosci. *21*, 788–797.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). Nat. Genet. *27*, 322–326.

Hendrich, B., Guy, J., Ramsahoye, B., Wilson, V.A., and Bird, A. (2001). Genes Dev. *15*, 710–723.

Inano, K., Suetake, I., Ueda, T., Miyake, Y., Nakamura, M., Okada, M., and Tajima, S. (2000). J. Biochem. *128*, 315–321.

Jackson-Grusby, L., Beard, C., Possemato, R., Tudor, M., Fambrough, D., Csankovszki, G., Dausman, J., Lee, P., Wilson, C., Lander, E., and Jaenisch, R. (2001). Nat. Genet. *27*, 31–39.

Jones, P.L., Veenstra, G.J., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J., and Wolffe, A.P. (1998). Nat. Genet. *19*, 187–191.

Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., and Bird, A. (1998). Nature *393*, 386–389.

Ng, H.H., Zhang, Y., Hendrich, B., Johnson, C.A., Turner, B.M., Erdjument-Bromage, H., Tempst, P., Reinberg, D., and Bird, A. (1999). Nat. Genet. *23*, 58–61.

Robertson, K.D., and Wolffe, A.P. (2000). Nat. Rev. Genet. *1*, 11–19.

Stancheva, I., Hensey, C., and Meehan, R.R. (2001). EMBO J. *20*, 1963–1973.

Van den Veyver, I.B., and Zoghbi, H.Y. (2000). Curr. Opin. Gen. Dev. *10*, 275–279.

Wan, M., Zhao, K., Lee, S.S., and Francke, U. (2001). Hum. Mol. Genet. *10*, 1085–1092.

Yusufzai, T.M., and Wolffe, A.P. (2000). Nucleic Acids Res. *28*, 4172–4179.