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The Odyssey of MeCP2 and Parental Imprinting

Janine M. LaSalle

Medical Microbiology and Immunology, Rowe Program in Human Genetics, School of Medicine,
One Shields Ave, University of California, Davis, CA, 95616, USA

Abstract

DNA methylation in mammals has long been implicated in the epigenetic mechanism of parental imprinting, in which selective expression of one allele of specific genes is based on parental origin. Methyl CpG binding protein 2 (MeCP2) selectively binds to methylated DNA and mutations in the *MECP2* cause the autism-spectrum neurodevelopmental disorder Rett syndrome. This review outlines the emerging story of how MeCP2 has been implicated in the regulation of specific imprinted genes and loci, including *UBE3A* and *DLX5*. The story of MeCP2 and parental imprinting has unfolded with some interesting but unexpected twists, revealing new insights on the function of MeCP2 in the process.

Keywords

parental imprinting; DNA methylation; neurodevelopment; methyl CpG binding protein; epigenetic

The non-Mendelian phenomenon of parental imprinting was originally discovered in the mid-1980's when mammalian embryos lacking genetic contributions from both parents failed to develop (1). Parental imprinting results in the selective expression of specific genes and genetic loci based on parental origin (2). Parental allele-specific DNA methylation is a hallmark of parentally imprinted regions and is required for the silencing of many imprinted genes (3). But until the mid-1990's, the mechanism of how DNA methylation resulted in gene silencing was unclear. Methyl CpG binding protein 2 (MeCP2) was the first described member of a family of proteins that selectively bind to methylated CpG sites and recruited the transcriptional silencer Sin3A and histone deacetylase (4–7). In 1999 *MECP2* mutations were found to be the genetic cause of the neurodevelopmental disorder Rett syndrome (8), an autism-spectrum disorder showing phenotypic overlap with the imprinted disorder Angelman syndrome. This discovery further stimulated the interesting question of whether MeCP2 was involved in parental imprinting mechanisms. But the resulting story has unfolded less like a paperback romance novel with a predictable plot and more like the classic tale *The Odyssey*, with unexpected obstacles to overcome and a soul-searching quest for a greater understanding of the true character of MeCP2 in the process.

Parental imprinting and neurodevelopment

Sexually reproducing eukaryotic organisms have diploid genomes comprised of one set of each chromosome from each parent. The original discovery of parental imprinting dates back to 1984, when pronuclear transplantation experiments in mouse demonstrated that simply having a diploid genome was not sufficient for development in mammals, as embryos lacking either maternal or paternal genetic contributions failed to develop (1). The mechanism of parental imprinting has been since explained by epigenetic marks placed on certain genetic loci during

the final stages of gametogenesis that serve to silence or “imprint” the expression of the gene in a parent-of-origin specific manner (8). A collated census of 83 mammalian transcriptional units have demonstrated imprinting, of which only 29 are imprinted in both mice and humans (9). Imprinted genes tend to be clustered together in chromosomal regions containing several distinctive features: parental allele-specific DNA methylation (3), asynchronous DNA replication (10), coordinated expression of multiple genes regulated by imprinting control regions (ICR) (11), allele-specific DNase hypersensitivity sites and histone modifications (12), and the presence of regulatory non-coding RNA transcripts (13).

While the evolutionary benefit for parental imprinting is an area of interesting speculation (14), parental imprinting appears to be especially relevant to the postnatal development of the mammalian nervous system. The human disorders Angelman syndrome (AS) and Prader-Willi syndrome (PWS) have demonstrated a biparental requirement for human chromosome 15 for normal brain development (15). PWS is primarily caused by paternal 15q11–13 deletion or maternal chromosome 15 uniparental disomy, resulting in mild mental retardation, hypogonadism, and early postnatal feeding problems, followed by predisposition to obesity. In contrast, AS is caused by maternal 15q11–13 deletion, paternal disomy, maternal methylation defects, or maternal mutations in *UBE3A*, encoding an E3 ubiquitin ligase. Mouse models of both disorders have supported the model of parental imprinting in this region being essential to normal brain development (16–19).

Although parthenogenetic mouse embryos die before implantation, both androgenetic (exclusively paternal) and gynogenetic (exclusively maternal) zygotes were used to make chimeric mice in which the parthenogenetic cells could be identified by a reporter gene (20, 21). Interestingly, gynogenetic cells were spatially restricted to the cortex, striatum, and hippocampus and the chimeras had enlarged brains and cortices. In contrast, androgenetic cells primarily localized to the hypothalamus and brainstem, and chimeras had relatively small brains and cortices. These results have suggested that the recently evolved parental differences in gene expression may have facilitated the rapid expansion of the brain in recent mammalian evolution. In addition, a recently described imprinted brain evolutionary theory has been described to extend the “extreme male brain” theory of autism to an “extreme imprinted brain” theory, in which autism is proposed to be a deficiency of highly evolved maternal influences on neocortical functions of social behavior, language, and executive function (22).

***MECP2*, Rett syndrome, and beyond**

Rett syndrome (RTT) is a severe neurodevelopmental disorder that falls under the current diagnostic category of pervasive developmental disorders (PDD) that also include autistic disorder, Asperger disorder, disintegrative disorder, and PDD not otherwise specified (23). All PDDs are characterized by impaired social and language development and the appearance of stereotyped repetitive behaviors. Unlike autism and Asperger’s which have a strong male bias, RTT primarily affects females by an X-linked dominant inheritance (8). Similar to autism, RTT has a postnatal onset, with apparently normal development in early infancy and apparent symptoms not appearing until 6–18 months of age. RTT is characterized by deceleration of head circumference, loss of purposeful hand movements, loss of language, ataxia, seizures, and respiratory dysfunction (24).

The discovery of *MECP2* mutations as the cause of RTT in 1999 opened up many avenues of understanding of the importance of epigenetics in postnatal brain development (8). Importantly, RTT is currently the only PDD with a known genetic cause. *MECP2* localizes to Xq28 and encodes the founding member of the family of methyl binding domain (MBD) proteins that selectively bind to methylated CpG dinucleotides (25). Several mouse models for RTT have been made using *cre*-targeted deletions or truncation mutations of *Mecp2* that demonstrate a

RTT-like phenotype specific to the postnatal central nervous system (CNS) (26–28). Although MeCP2 was originally presumed to be a ubiquitous transcriptional repressor of methylated genes, both RTT and the mouse models have demonstrated that MeCP2 deficiency results in a selective defect in postnatal neuronal maturation. The dynamic developmental expression of MeCP2, in which highest expression is limited to mature postnatal neurons, is the most likely explanation for the tissue and developmental-stage specific manifestation of *MECP2/Mecp2* mutations (29–33).

In addition to the clear relevance to RTT, in which 96% of patients with classic symptoms have detectable *MECP2* mutations or deletions (34), *MECP2* has been implicated in a wider range of neurodevelopmental disorders. Although *MECP2* mutations were originally thought to be lethal in males, a number of reports of males with *MECP2* mutations and RTT, Angelman syndrome, X-linked mental retardation, or severe neonatal encephalopathy have been described (35–39). *MECP2* duplications were also recently described to be a frequent cause of severe X-linked mental retardation in males (40), consistent with transgenic mouse models that have demonstrated the detrimental neurological effect of *Mecp2* overexpression (41,42). Furthermore, autism brain samples were found to exhibit *MECP2* expression defects by multiple transcriptional and post-transcriptional pathways (43).

The dogma challenged

With the clinical relevance of *MECP2* to neurodevelopmental disorders clearly established, a logical next step in the story was to determine if MeCP2 was required to regulate downstream gene targets known to be regulated by DNA methylation, namely parentally imprinted genes. The requirement for DNA methylation and parental imprinting has been clearly demonstrated in embryos deficient in the DNA methyltransferase genes, *Dnmt1*, *Dnmt1o*, *Dnmt3a*, *Dnmt3b*, and *Dnmt3l* (summarized in Table 1) (44–47). In addition, the histone H3 methyltransferase G9a was essential for normal *Snrpn* imprinting in embryonic stem cells (48). Thus it was not a long stretch to hypothesize that the MBD proteins that read the methylation marks and repress methylated constructs *in vitro* would also be required for maintaining imprinted expression.

An unexpected twist in the MeCP2 story came with the failure of most imprinted genes to be repressed by either MeCP2 or MBD2 (Table 1). In human RTT patients, several imprinted genes showed maintenance of monoallelic expression in *MECP2*-mutant-expressing clonal T lymphocytes and post-mortem brain (49). In *Mecp2*-null mouse brain, imprinted expression was also faithfully maintained for five imprinted genes (50), similar to what was observed in MBD2-null mice (51). And while the imprinted gene *H19* was described as a “bona-fide” MeCP2 target gene because of the direct binding of MeCP2 and a 2-fold increased expression in *Mecp2*-null cells (52), the increased expression could not be explained by a failure to silence the paternal *H19* allele in the absence of MeCP2 (43,49). In addition to imprinted genes, a nonimprinted gene regulated by methylation, *IFNG*, and LINE-1 retrotransposon expression were apparently unaltered in *MECP2*-mutant T cell clones (49).

Several genome-wide expression profiling studies in RTT and *Mecp2*-null mice also turned up the unexpected finding that MeCP2 does not appear to be required for global transcriptional repression in brain or other tissues (53–55). Although there has been very little overlap between the MeCP2 dysregulated gene lists from several different studies, some imprinted genes were apparently dysregulated, including *IGF2* and *GNAS* in RTT lymphoblasts (53) and *CDKN1C* in a differentiated, MeCP2 blocked neuronal cell line (56). One lesson learned in the quest for MeCP2 target genes may be that the gene targets found may completely depend on the cellular and developmental context being studied. For instance, *Bdnf* was upregulated in *Mecp2*-deficient neuronal cultures (57), but downregulated in *Mecp2*-deficient brain (58);

glucocorticoid responsive genes were upregulated in *Mecp2*-deficient mouse brain (59), but not cell lines; and a family of inhibitors of differentiation genes (*ID1-4*) were identified as MeCP2 targets during the maturation of a neuronal cell line, but were only transiently transcriptionally dysregulated in *Mecp2*-deficient brain (56). Thus, the lack of reproducible results for obtaining and validating MeCP2 target genes from different laboratories may not be totally surprising.

An epic battle over *Ube3a*

As any interesting plot development involves conflict, so has been the case of the discrepant results involving *Ube3a* being battled out on the pages of *Human Molecular Genetics* over the past year. At stake is a potential explanation for the overlapping phenotype between Rett and Angelman syndromes as well as a potential MeCP2 target gene that shows selective imprinting limited to postnatal neurons and is essential for normal brain development. However, the *UBE3A/Ube3a* locus is no easy battleground. In addition to *UBE3A/Ube3a*'s tissue and developmental specific imprinting (60–62), the gene has at least 5 alternatively spliced transcripts as well as multiple antisense transcripts (*Ube3a-as*) that originate over 0.5 MB from the *Ube3a* coding region (63–65) that include *SNRPN* as well as a large family of snoRNA genes (66). And despite the known function of *UBE3A* as an E3 ubiquitin ligase (67), known mutations in AS (68,69), and two available animal models (17,18), there is still no real understanding of the essential role of *UBE3A* in neuronal development.

In early 2005, Samaco *et al.* demonstrated a 1.5–2 fold reduction of *Ube3a* expression in two different strains of adult *Mecp2*-deficient mice by quantitative immunofluorescence, immunoblot, TaqMan RT-PCR, and *in situ* hybridization (50). Curiously, the reduced *Ube3a* expression in *Mecp2*-deficient mice could not be explained by a change in imprinted expression of either *Ube3a* sense or antisense transcripts, increased expression of *Ube3a-as*, or MeCP2 binding directly to the putative *Ube3a* promoter. Significantly reduced *UBE3A* expression was also observed in RTT and autism post-mortem brain. Three months later, Makedonski *et al.* also reported reduced *UBE3A* expression in RTT brain and neonatal *Mecp2*-deficient brain, but proposed that this was due to aberrant acetylation and expression of the maternal *Ube3a* antisense transcript (70). Most recently, Jordan and Francke reported that *Ube3a* expression was not altered in two strains of *Mecp2*-deficient brain by quantitative real-time RT-PCR and immunoblot, using *Ube3a* deletion mice as a control (71).

So what are some possible explanations for these discrepant *Ube3a* results? The complexity of the *Ube3a* locus and the differences in assays, reagents, and developmental time points used by the three studies are the most likely reasons. The difference in the *Ube3a-as* imprinting results could be explained by the differences in using intra- versus inter-species crosses for determining allelic expression, as the interspecies cross of *M. musculus* and *M. spretus* used by Makedonski *et al.* (70) exhibits widespread disruption of imprinted genes (72). Despite evidence for MeCP2 binding to the *Snrpn* imprinting control region that controls *Ube3a-as* transcription (50,73), unaltered *Snrpn* expression in *Mecp2*-deficient mice has been shown in two independent laboratories (50,74) and Makedonski *et al.* did not show evidence for increased expression of *Ube3a-as* in *Mecp2* deficient brain (70). Therefore, the aberrant *Ube3a-as* imprinting model proposed by Makedonski *et al.*, while somewhat satisfying, is based on scant evidence. The failure of Jordan and Francke (71) to detect significant differences in *Ube3a* expression in *Mecp2*-deficient mice is likely due to the neonatal time point used, as the phenotype in *Mecp2* deficient mice does not manifest until 3–4 weeks of age (26,27). Although 8-week old mice also failed to show significant differences in *Ube3a* expression (71), differences in primers and normalization controls in quantitative RT-PCR assays may explain the discrepancies with the two prior studies (50,70). Complications in separating out sense from antisense strands of *Ube3a* are also a major concern with real-time RT-PCR

analyses. Although a nonsignificant decrease in *Ube3a* was observed by quantitative TaqMan RT-PCR in the Samaco *et al.* study, *in situ* hybridization using single-stranded riboprobes to differentially detect sense and anti-sense strands showed a significant reduction in the *Ube3a* sense but not antisense transcript (50).

Since no MeCP2 target gene has yet been found that stands up to the criteria of being reproducibly dysregulated in multiple laboratories and conditions, the battle over *Ube3a* appears to reflect the inability to define what constitutes a “bona fide” MeCP2 target gene. *Ube3a* does not appear to fit the expectations of a direct target of MeCP2 because the promoter is unmethylated and unbound by MeCP2 and the expression is reduced by *Mecp2* deficiency rather than increased. However, even indirect reduced *UBE3A* expression in RTT patients is intriguing and significant because of the phenotypic overlap with AS and the potential for further understanding the molecular pathogenesis of RTT. Importantly, *UBE3A* was significantly decreased in RTT postmortem brain by quantitative RT-PCR (Hogart *et al.*, in preparation), supporting the two prior studies showing decreased *UBE3A* protein expression in RTT brain (50,70).

Loops untangle the plot

Also in 2005, Horike *et al.* used an alternative approach to identify MeCP2 target genes by cloning and sequencing DNA fragments following chromatin immunoprecipitation of mouse brain (74). This approach successfully identified an interesting imprinted MeCP2 target locus on mouse chromosome 6 (syntenic to human chromosome 7q21–22) containing two *Dlx* genes that regulate the production of γ -aminobutyric acid (GABA). Both *Dlx5* and *Dlx6* as well as the neighboring imprinted gene *Peg10* were upregulated in MeCP2 deficient compared to wild-type mouse brain. Interestingly, the CpG islands of both *Dlx5* and *Dlx6* were unmethylated, but MeCP2 instead bound to methylated sequences within the introns and was necessary for the formation of a silent chromatin loop between the two genes. While the imprinted expression of *DLX5* was apparently disrupted in 3 of 4 RTT lymphoblast lines (74), the variable imprinting of *DLX5* in 13/15 normal human lymphoblast samples (75) suggests that *MECP2* mutations may not be entirely responsible for biallelic *DLX5* expression in RTT lymphoblasts. In addition, *Dlx5* is not imprinted in mouse brain where the loss of silent chromatin looping and increased *Dlx5* expression was observed with *Mecp2* deficiency (74). Therefore, the loss of silent chromatin looping rather than loss of imprinted expression appears to be the major cause of dysregulated *Dlx5/Dlx6* expression in *Mecp2* deficient mouse brain.

Chromatin loops have also been recently investigated as a way of explaining parental allele-specific differences in the imprinted locus containing *Igf2* and *H19* (76). On the maternal allele, the ICR of *H19* is unmethylated and the chromatin insulator CTCF interacts with two flanking sequences of *Igf2*, positioning *Igf2* in a silent chromatin loop (77). Since the binding of CTCF is sensitive to methylation and the *H19* ICR is methylated on the paternal allele, *Igf2* is activated by the absence of the loop. It is intriguing to consider that MeCP2 may be serving a similar function by selectively looping methylated sequences in imprinted regions and thereby indirectly regulating gene expression levels without being essential for maintenance of parental imprints.

The Horike *et al.* study therefore introduced a novel mechanism by which MeCP2 may exhibit long-range regulation of gene expression at imprinted loci. Unlike the previously proposed enzymatic or structural models proposed for MeCP2 (78), the looping model could potentially explain why MeCP2 was originally identified as an AT-rich binding protein (ARBP) and nuclear matrix component in chicken (79). A more recent study has shown that MeCP2 has highest affinity to oligonucleotides with single methylated CpG sites adjacent to AT runs, frequently found at the base of chromatin loops (80). The looping model can also potentially

explain why MeCP2 was shown to function at a long range of >500bp from the transcription start site in early transfection experiments (81). In addition, a recent study has shown that MeCP2 associates with the RNA splicing factor YB-1 and regulates alternative splicing of multiple genes (82). Since RNA splicing factors are major components of the nuclear matrix where chromatin loops are localized (83), this new “moonlighting” function for MeCP2 may begin to explain the difficulty in finding transcriptionally repressed gene targets of MeCP2 by genome-wide screening approaches.

Disrupted neighbors

In both the Horike *et al.* and Samaco *et al.* publications, an interesting finding in addition to dysregulated imprinted genes was that some non-imprinted genes within the imprinted domains showed altered expression in *Mecp2*-deficient brain (50,74). In the *Dlx5* locus, the biallelically expressed genes *Bet1*, *Cas1*, and *Pdk4* were all downregulated in *Mecp2*-deficient brain (74). In the PWS/AS locus, *GABRB3/Gabrb3* was found to be significantly downregulated in *Mecp2*-deficient mouse and human RTT brain (50). While *Gabrb3* is biallelically expressed in mouse and the allelic expression was unaffected by *Mecp2* deficiency, *GABRB3* protein deficiency was common to RTT, AS, and autism brain. Since *GABRB3* encodes an abundant GABAA receptor β subunit (84) and *Mecp2*-deficient brain is characterized by defects in the GABA mediated inhibitory transmission (85), expression defects in *GABRB3* may explain the seizures common to all three disorders. Perhaps long-range chromatin looping of imprinted chromosomal domains by MeCP2 affects other non-imprinted genes within imprinted domains that may be relevant to the pathogenesis of RTT.

Kissing chromosomes

In addition to long range *cis*-acting regulation by chromatin looping and imprinting control regions, *trans* effects have been observed in several different imprinted loci, suggesting that maternal and paternal alleles can cooperatively interact. Transient *trans*-sensing interactions have recently been demonstrated to be necessary for the chromosome counting and choice stages of X chromosome inactivation in females (86,87). While the precise role for homologous *trans* interactions within imprinted regions is not yet apparent, evidence for *trans* effects on methylation and gene expression have been observed in at least three different imprinted loci (88–90). Both X chromosome inactivation and parental imprinting involve regulation by antisense transcripts and *trans* effects have been demonstrated between *Ube3a* sense and antisense transcripts in mouse brain (65).

Homologous pairing of oppositely imprinted chromosomes has been observed for the imprinted 15q11–13 and 11p15 in cycling lymphocytes (91). More recently, a developmental increase in homologous 15q11–13 pairing of the *GABRB3* locus was observed in human cortical neurons and neuronal cultures (73). Homologous pairing of *GABRB3* loci was deficient in RTT, AS, and autism brain and partially inhibited by blocking MeCP2 binding in neuronal cultures, suggesting that MeCP2 plays a role in the interaction of maternal and paternal homologs at the *GABRB3* locus.

The saga continues

The stage is now set to answer more questions about MeCP2 and parental imprinting. To complete the list in Table 1, it would be useful to test imprinted genes in knockout mice of the other MBD containing genes to determine if perhaps one or more member of this family is required for maintaining the silent imprint. An alternate possibility may be that DNA methylation is required for establishing the parental imprints, as observed in the *Dnmt*-deficient mice (44,45), but not the maintenance of the silencing mark, which may be instead obtained by histone modifications. Determining imprinted gene expression in mice with genetically

modified histones or histone methyltransferases may help to illuminate whether DNA or histone methylation is more important for imprint maintenance.

Another important question to address is how MeCP2 may be regulating the level of expression of some imprinted genes and neighboring non-imprinted genes without necessarily altering imprinted expression. To further test the long-range chromatin looping model, the specific MeCP2 binding sites need to be defined and the effect of MeCP2 deficiency on chromatin loop structure determined, as was performed in the *Dlx5* locus (74). Recent studies identifying alternative MeCP2 protein isoforms (92,93) and post-translation modifications (57,94) are probably just the beginning of understanding a molecule that is likely to have functional dynamics as complex as histone core proteins. Certainly, MeCP2 needs to be better understood through careful investigations into post-translational modifications, associating factors, and alternate isoforms. Rationally-designed genome-wide screens are expected to continue to uncover new and important MeCP2 target genes. However, more global approaches to understand how MeCP2 may indirectly regulate gene expression through changes in nuclear structure and organization may also be equally important. The currently murkiness of the MeCP2 field is expected to become clearer as these studies are performed. Perhaps then, MeCP2 and imprinting questions can be addressed with a fresh and enlightened perspective. Stay tuned for an exciting denouement!

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Abbreviations

MeCP2	methyl CpG binding protein 2
AS	Angelman syndrome
PWS	Prader-Willi syndrome
RTT	Rett syndrome, PDD, pervasive developmental disorder
PDD	pervasive developmental disorder
MBD	methyl binding domain
CNS	central nervous system
RT-PCR	reverse transcriptase polymerase chain reaction
GABA	gamma aminobutyric acid

Table 1
Requirement for modifiers of DNA and histone methylation for maintaining parental imprints

		Imprinted expression altered?	
		Yes	No
DNA methyltransferase	DNMT1	<i>H19, Igf2, Igf2r</i> (47)	
	DNMT1o	<i>H19, Snrpn</i> (45)	<i>Igf2, Igf2r</i> (45)
	DNMT3A	<i>H19, Rasgrf1, Igf2r, Peg1, Peg3, Snrpn</i> (44,46)	
	DNMT3B	<i>H19, Rasgrf1, Igf2r, Peg1, Peg3, Snrpn</i> (44,46)	
	DNMT3L	<i>Igf2r, Peg1, Peg3, Snrpn</i> (44)	
Methyl CpG binding protein (MBD)	MBD2		<i>Igf2r, Peg1, Peg3, Zim1, Snrpn, Znf127</i> (51)
	MeCP2	<i>DLX5</i> (74), <i>Ube3a-as</i> (70)	<i>SNRPN, IPW, IGF2, NDN, H19</i> (49) <i>Ube3a, Ube3a-as, Snrpn, Rasgrf1, H19</i> (50)
Histone methyltransferase	G9a	<i>Snrpn</i> (48)	