Methyl-CpG-binding protein 2 mutations in Rett syndrome

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The X-linked methyl-CpG-binding protein 2 gene (*MECP2)* encodes a protein that links DNA methylation to transcriptional repression mediated by histone deacetylases. Mutations in *MECP2* have been found in 76% of classic Rett syndrome patients. Favourable nonrandom X chromosome inactivation ameliorates the phenotype.

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Abbreviations

5mc	position 5 of cytosine
ES	embryonic stem
MBD	methyl-binding domain
MeCP2	methyl-CpG-binding protein 2
RTT	Rett syndrome
TRD	transcriptional repression domain
XCI	X chromosome inactivation

Introduction

The aetiology of Rett syndrome (RTT) has been subject to debate since the syndrome was first described by Andreas Rett in 1966 [1–4]. The exclusive occurrence of classic RTT in females, the existence of a few familial cases with documented inheritance through the maternal line, and concordance in monozygotic twins all suggested a genetic origin [5,6•,7•]. Further evidence supported X-linked dominant inheritance with male lethality: three males born into RTT families suffered severe neonatal encephalopathy and died in infancy [6•,7•,8,9]. Definitive confirmation of this hypothesis was finally provided in 1999 by the discovery that several different mutations in *MECP2*, the gene encoding methyl-CpG-binding protein 2 (MeCP2), were responsible for roughly a third of classic RTT cases [10••,11••]. Recent studies have augmented this figure to 76% [12••].

This discovery provided an explanation for the restriction of classic RTT to females, broadened the range of phenotypes related to RTT, and suggested a basis for phenotypic variability in classic RTT. It also raised many new questions. MeCP2 participates in transcriptional silencing via DNA methylation, but which genes are regulated by its transcriptional activity? Does the type of mutation influence the phenotype? How important is X chromosome inactivation (XCI) in classic RTT? Does the period of early normal development in RTT girls offer an opportunity for therapeutic intervention? While some of these questions await dedicated pursuit, others have already been answered. In this review, we will describe the features of Rett syndrome and what has been learned about its genetic basis and pathogenesis since the discovery of the disease-causing mutations.

Features of Rett syndrome

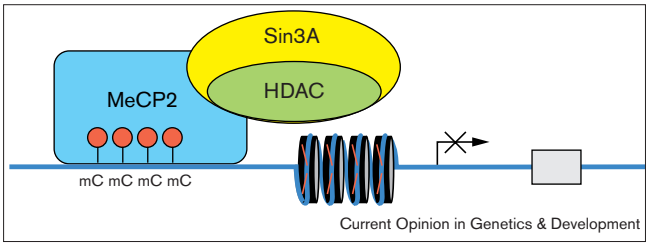
Rett syndrome, one of the leading causes of mental retardation and autistic behaviour in girls, is a neurodevelopmental disorder affecting 1/10,000–1/15,000 females [2,13,14]. These infants have apparently normal development until 6–18 months, after which they undergo a period of rapid regression with loss of purposeful hand use, deceleration of head growth and onset of repetitive, stereotyped hand movements. They develop gait ataxia and apraxia, autistic features, seizures and respiratory dysfunction (episodic apnoea and/or hyperpnoea), and they have decreased somatic growth [15–17]. This period of rapid deterioration is followed by a stagnation phase, which lasts throughout adulthood [2,18,19]. Some female patients who do not manifest all the typical features are considered to have a variant, usually milder, form of the disease [20,21]. Prior to the recent gene discovery, there were no specific laboratory or pathological markers for RTT; diagnosis had to be based solely on the presence or absence of a set of clinical features in a relatively advanced stage of the disease [22,23]. Brain imaging and pathological studies of RTT patients have revealed some degree of localized cerebral atrophy, characterised by small neuronal size and reduced dendritic arborisation, but no neuronal loss [24–26]. These were early indications that RTT was a neurodevelopmental, rather than a neurodegenerative, process [24,25,27].

Structure and function of MECP2

MECP2 maps between *L1CAM* and the *RCP/GCP* loci in Xq28 and undergoes X inactivation [28,29]. MeCP2 is an abundant, ubiquitously expressed nuclear protein of 486 amino acids, encoded in three exons [28,30–32]. The third exon of the *MECP2* gene contains a large (>8.5 kb) 3′-untranslated region (UTR), with several polyadenylation sites that enable the generation of multiple transcripts of different lengths. The human and mouse 3′-UTR sequences have at least eight regions of high sequence similarity that may be important for transcript stability and post-transcriptional regulation [33••].

MeCP2 is a chromosome-binding protein that specifically binds with its methyl-binding domain (MBD) to symmetrically positioned CpG dinucleotides, methylated at position 5 of cytosine (5mC). MeCP2 can bind throughout the chromosome, but it preferentially associates with dense 5mC-rich heterochromatin [30]. Methylation of CpG

Figure 1



Normal function of MeCP2. MeCP2 (light blue) binds methylated cytosine residues (mC, red circles) in CpG islands and recruits Sin3A (yellow) and histone deacetylase (HDACs, green). Deacetylation of the histone tails compacts the chromatin and silences transcription. Deacetylated histone tails are represented as red lines linking the nucleosomes to the DNA strand (in dark blue) over the nucleosomes (black rectangles).

dinucleotides in the mammalian genome is important for transcriptional silencing in processes such as X-inactivation and imprinting, in the immobilisation of transposons and the control of tissue-specific gene expression [34]. When MeCP2 binds to methylated CpG islands, its transcriptional repression domain (TRD) recruits a co-repressor complex containing Sin3A and histone deacetylases (HDAC); the tails of core histones H3 and H4 in the nucleosomes are deacetylated by the HDACs [35••,36••,37]. This leads to compaction of the chromatin, making it inaccessible to components of the transcriptional machinery and resulting in stable repression of the target gene [38] (Figure 1).

Types of MECP2 mutations found in RTT

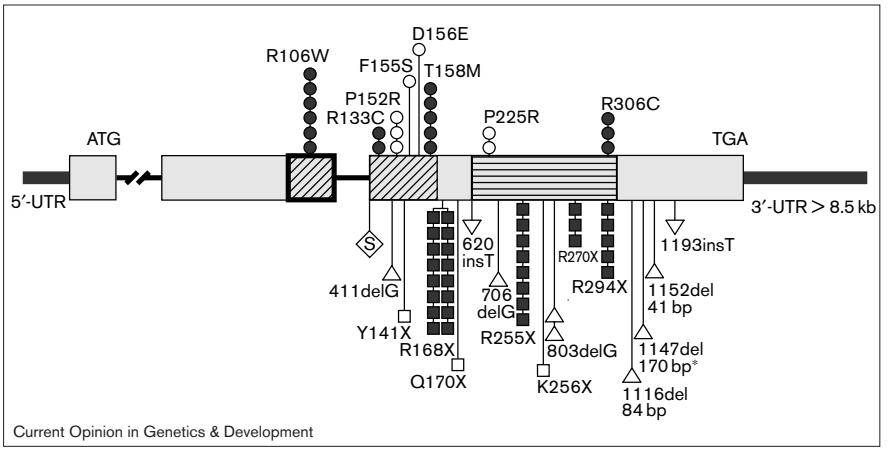
At this time, mutations in *MECP2* have been found in 76% of sporadic RTT patients and 45% of familial cases. Figure 2 lists all 68 *MECP2* mutations reported in Rett syndrome to date [10••–12••]. Twenty three (34%) are missense mutations, of which 18 cluster in the MBD. Forty five (66%) are nonsense and frameshift mutations, predicted to truncate the protein. All except three of these are distal to the MBD, affecting the TRD and carboxyl terminus of the protein. In total, 24 different *de novo* mutations have been described, 11 of which recurred in independent meioses. Consistent with the sporadic occurrence of RTT, the vast majority of the mutations are *de novo*. Of these, 48 (71%) were C to T transitions at CpG mutation hotspots.

Three mutations are predicted to truncate the MBD. In one patient, the putative truncating mutation affects the acceptor splice site of exon 3. Only the wild type transcript was detectable in lymphoblast mRNA from this patient; either she has a nonrandom XCI pattern, or the RNA is unstable. The first possibility could not be confirmed because the patient was uninformative for markers used in XCI studies. The other two mutations (Y141X, 411delG) are distal to the DNA-binding surface of the MBD, and the affected patients have a tendency to nonrandom XCI in genomic DNA from peripheral blood leukocytes or lymphoblastoid cell lines [11••,12••]. These data strongly suggest that mutations that truncate MeCP2 early are not compatible with survival unless there is nonrandom XCI (see below). Interestingly, we also found three multiple nucleotide deletions (41–170 bp) distal to the region that encodes the TRD. This area contains a number of (quasi) palindromic repeats that may make the region vulnerable to such deletions [39]. Despite the fact that RTT samples have not yet been completely evaluated for larger deletions, or for noncoding region mutations, the high proportion of patients with *MECP2* mutations makes it unlikely that there are other major loci for RTT.

Influence of X chromosome inactivation

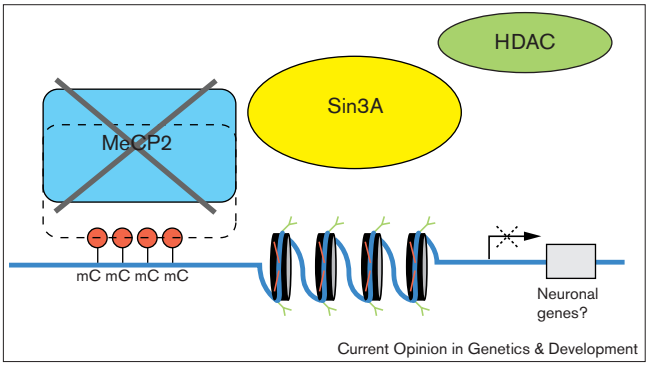
Previous studies found most RTT patients to have a random XCI pattern, but some reports of nonrandom XCI exist

Figure 2



MeCP2 mutations in Rett syndrome. Diagrammatic representation of the *MECP2* gene. Exons are represented as light grey boxes, noncoding regions in dark grey, the MBD in diagonal lines and the TRD in horizontal lines. Circles above the gene represent missense mutations; truncating mutations are shown below the gene; squares represent nonsense mutations; triangles represent frameshift mutations; S is the splicing mutation. Missense and nonsense mutations are referred to by their position in the amino acid sequence (single-letter nomenclature). Deletions (del) and insertions (ins) are indicated by their nucleotide sequence position. 'X' indicates a stop codon. Recurrent mutations are indicated by the number of symbols. Mutations at CpG dinucleotides are in filled circles or squares. The asterisk indicates an insertion of 3 bp in the frameshift mutation 1147del170 bp. Single letter amino acid codes are shown throughout.

Figure 3



Putative influence of RTT-causing mutations on the function of Mecp2. (The same colour scheme and symbols are used as Figure 1) Mutated Mecp2 protein may not properly bind or recruit Sin3A and HDACs (histone deacetylases), leading to partial loss of transcriptional repression. The hatched outline indicates possible absent or altered binding of Mecp2 to methylated cytosine residues (mC); the large 'X' indicates decreased or absent function of Mecp2. The small arrow with the hatched cross indicates a possibly reactivated promoter of downstream target genes. The question mark denotes uncertainty about the identity of the Mecp2 target genes affected in Rett syndrome.

[40–44]. Because the *MECP2* gene undergoes X inactivation [28], we sought to determine how XCI patterns influence the phenotypic expression of RTT mutations. We determined that 91% of 34 patients with classic RTT and known *MECP2* mutations have random XCI [12••]. Nonrandom XCI has been found in three mildly symptomatic or asymptomatic carrier females (two of whom have known mutations in *MECP2*) and in the unaffected member of a pair of monozygotic twins discordant for the Rett phenotype [4,6•,11••,40,45]. Preferential inactivation of the X chromosome with the mutated *MECP2* gene thus protects against the deleterious effects of the mutations. Moreover, the nonrandom XCI mitigates the otherwise severe phenotype expected in the three patients with truncating mutations.

Complete or partial loss of *MeCP2* function in the pathogenesis of Rett syndrome?

An early truncating mutation of murine *Mecp2* that prevented protein formation was generated using homologous recombination in embryonic stem (ES) cells [31]. Male ES cells carrying this mutation are viable and able to differentiate in culture, but when they are injected into blastocysts and transferred to pseudopregnant females, chimeric embryos with a high contribution from mutant ES cells die between embryonic day 8.5 and 12.5. Their severe malformations suggest that *Mecp2* is essential for gastrulation [31]. It is noteworthy that this phenotype resembles that of embryos deficient for maintenance DNA methyltransferase (*Dnmt1*) [46] — DNA methylation could play an important role in development.

The chimeric male embryos are mosaic for cells with normal and abnormal *Mecp2* function, similar to human females with RTT who have random XCI and, in contrast to the mice, usually survive. Early truncations, however, such as the one generated in mice, are rarely seen in humans. If present, they are associated with nonrandom XCI [11••,12••]. This suggests not only that these mutations are incompatible with survival in humans as well as mice, but that the *MECP2* mutations identified to date lead to partial rather than complete loss of MeCP2 function (Figure 3). Proof for this hypothesis is lacking, however, and alternative possibilities must be explored. MeCP2 target genes may differ between humans and mice, or there might be differences in MeCP2's role during central nervous system (CNS) development. Because XCI leads to a cell-autonomous inactivation of this nuclear protein, a dominant-negative effect is less likely but remains conceivable. MeCP2 belongs to a family of methyl-binding domain-containing proteins [47], at least three of which are members of transcriptional repressor complexes whose functions may overlap with those of the Mecp2 complex [34]. A mutated MeCP2 molecule with altered DNA-binding kinetics, or one that can bind 5mC but cannot repress transcription, could thus interfere with the normal function of these other MBD proteins by blocking their access to 5mC residues.

MeCP2 and the central nervous system phenotype of RTT

One would expect mutations in a ubiquitous protein to affect multiple bodily systems, but classic RTT manifests itself predominantly in the CNS, even though somatic growth failure, cardiac and intestinal abnormalities hint at a wider impact. The tissue-specific differences in the expression levels of the *MECP2* alternative transcripts may account, in part, for the specificity of the RTT phenotype: the 1.8 and 5 kb transcripts are highest in foetal liver, although transcripts of all sizes, including a 7.5 kb band, are visible on Northern blots of all tissues. Interestingly, MECP2 is highly expressed in foetal brain, where the largest 10.1 kb transcript containing the longest 3′-UTR is the predominant isoform [28,33••]. The developing brain may be more dependent on MECP2 for transcriptional silencing than other tissues, and different MECP2 isoforms could have an important regulatory function in this process.

It remains unknown which genes are targeted by MeCP2 activity. Is MeCP2 more important for overall reduction of transcriptional noise or for highly specific regulation of expression of a relatively small number of genes during development [34]? MeCP2's global chromosomal binding implies the first possibility, but the regulated expression of its alternative transcripts and the RTT phenotype support the latter. To settle this issue it will be necessary to compare gene expression levels in various tissues between animal models for Rett syndrome and their wild-type littermates, or between patients with mutations and unaffected individuals. It is reasonable to expect that at least some genes altered by MeCP2 are critical for neuronal development, since small neuronal size and

reduced dendritic arborisation are commonly found in brains from RTT patients [24–26].

Conclusions

Mutations in *MECP2* are responsible for at least 76% of sporadic Rett syndrome cases. Although we lack conclusive evidence, we can infer from the available data that RTT results from partial, rather than complete, loss of function of MeCP2. Partial inactivation of MeCP2 is predicted to cause inappropriate expression of its target genes during development and in tissues where these genes are normally silenced. Most patients with classic RTT and known *MECP2* mutations have random XCI; favourable nonrandom XCI mitigates the consequences of *MECP2* mutations. Furthermore, *MECP2* mutations lead to a broader array of phenotypes than previously suspected, ranging from neonatal encephalopathy in males to very subtle learning deficits in healthy adult females. As *MECP2* is only one member of a family of genes that play a role in DNA methylation-dependent transcriptional repression, it will be interesting to find out whether mutations in these other genes cause developmental disorders (such as autism) which share some features with RTT.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Rett A: **Uber ein zerebral-atrophisches Syndrome bei Hyperammonemie.** *Wien Med Wochenschr* 1966, **116**:723-726. [Title translation: On an unusual brain atrophy syndrome with hyperammonemia in childhood.]

2. Hagberg B, Aicardi J, Dias K, Ramos O: **A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases.** *Ann Neurol* 1983, **14**:471-479.

3. Martinho PS, Otto PG, Kok F, Diament A, Marques-Dias MJ, Gonzalez CH: **In search of a genetic basis for the Rett syndrome.** *Hum Genet* 1990, **86**:131-134.

4. Migeon BR, Dunn MA, Thomas G, Schmeckpeper BJ, Naidu S: **Studies of X inactivation and isodisomy in twins provide further evidence that the X chromosome is not involved in Rett syndrome.** *Am J Hum Genet* 1995, **56**:647-653.

5. Zoghbi H: **Genetic aspects of Rett syndrome.** *J Child Neurol* 1988, **3**:S76-S78.

6. • Sirianni N, Naidu S, Pereira J, Pillotto RF, Hoffman EP: **Rett syndrome: confirmation of X-linked dominant inheritance, and localization of the gene to Xq28.** *Am J Hum Genet* 1998, **63**:1552-1558.

The last of a series of exclusion mapping studies that finally narrowed the location of the gene to Xq28.

7. • Schanen NC, Kurczynski TW, Brunelle D, Woodcock MM, Dure LS 4th, Percy AK: **Neonatal encephalopathy in two boys in families with recurrent Rett syndrome.** *J Child Neurol* 1998, **13**:229-231.

This is a report of a severely affected male in a pedigree of familial RTT. Further evidence is provided for X-linked dominant inheritance of RTT, but, more importantly, this paper suggests that RTT is not uniformly lethal in males. This was subsequently proven by the demonstration of a mutation in the patient described in Wan *et al.* [11••].

8. Schanen C, Francke U: **A severely affected male born into a Rett syndrome kindred supports X-linked inheritance and allows extension of the exclusion map.** *Am J Hum Genet* 1998a, **63**:267-269.

9. Webb T, Clarke A, Hanefeld F, Pereira JL, Rosenbloom L, Woods CG: **Linkage analysis in Rett syndrome families suggests that there may be a critical region at Xq28.** *J Med Genet* 1998, **35**:997-1003.

10. •• Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY: **Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2.** *Nat Genet* 1999, **23**:185-188.

This is the first report that mutations in the X-linked *MECP2* gene are responsible for Rett syndrome. Mutations were found in 25% of patients and a loss-of-function mechanism is suggested.

11. •• Wan M, Lee SS, Zhang X, Houwink-Manville I, Song HR, Amir RE, Budden S, Naidu S, Pereira JL, Lo IF *et al.*: **Rett syndrome and beyond: recurrent spontaneous and familial *MECP2* mutations at CpG hotspots.** *Am J Hum Genet* 1999, **65**:1520-1529.

This paper describes mutations in 40% of patients with Rett syndrome and draws attention to the high rate of *de novo* C→T transitions at CpG hotspots, the variability of the phenotypic spectrum of *MECP2* mutations (from mild learning disability in females with favourable XCI to severe neonatal encephalopathy in males) is highlighted.

12. •• Amir RE, Van den Veyver IB, Schultz R, Malicki DM, Tran CQ, Dahle EJ, Philippi A, Timar L, Percy AK, Motil KJ *et al.*: **Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes.** *Ann Neurol* 2000, in press.

This paper establishes that the majority (76%) of RTT patients have mutations in *MECP2*, identifies 14 novel mutations, and correlates phenotype with each mutation, taking into account XCI.

13. Hagberg B: **Rett's syndrome: prevalence and impact on progressive severe mental retardation in girls.** *Acta Paediatr Scand* 1985, **74**:405-408.

14. Hagberg B, Hagberg G: **Rett syndrome: epidemiology and geographical variability.** *Eur Child Adolesc Psychiatry* 1997, **6**:5-7.

15. Witt Engerstrom I: **Age-related occurrence of signs and symptoms in the Rett syndrome.** *Brain Dev* 1992, **14 (suppl)**:S11-S20.

16. Glaze DG, Schultz RJ, Frost JD: **Rett syndrome: characterization of seizures versus non-seizures.** *Electroencephalogr Clin Neurophysiol* 1998, **106**:79-83.

17. Naidu S: **Rett syndrome: a disorder affecting early brain growth.** *Ann Neurol* 1997, **42**:3-10.

18. Budden SS: **Rett syndrome: habilitation and management reviewed.** *Eur Child Adolesc Psychiatry* 1997, **6**:103-107.

19. Motil KJ, Schultz RJ, Browning K, Trautwein L, Glaze DG: **Oropharyngeal dysfunction and gastroesophageal dysmotility are present in girls and women with Rett syndrome.** *J Pediatr Gastroenterol Nutr* 1999, **29**:31-37.

20. Hagberg BA: **Rett syndrome: clinical peculiarities, diagnostic approach, and possible cause.** *Pediatr Neurol* 1989, **5**:75-83.

21. Zappella M, Gillberg C, Ehlers S: **The preserved speech variant: a subgroup of the Rett complex: a clinical report of 30 cases.** *J Autism Dev Disord* 1998, **28**:519-526.

22. Hagberg B, Witt-Engerström I: **Rett syndrome: a suggested staging system for describing impairment profile with increasing age towards adolescence.** *Am J Med Genet* 1986, **24**:47-59.

23. Trevathan E, Moser H: **Diagnostic criteria for Rett syndrome. The Rett Syndrome Diagnostic Criteria Work Group.** *Ann Neurol* 1988, **23**:425-428.

24. Oldfors A, Sourander P, Armstrong DL, Percy AK, Witt-Engerstrom I, Hagberg BA: **Rett syndrome: cerebellar pathology.** *Pediatr Neurol* 1990, **6**:310-314.

25. Armstrong D, Dunn JK, Antalffy B, Trivedi R: **Selective dendritic alterations in the cortex of Rett syndrome.** *J Neuropathol Exp Neurol* 1995, **54**:195-201.

26. Bauman ML, Kemper TL, Arin DM: **Microscopic observations of the brain in Rett syndrome.** *Neuropediatrics* 1995, **26**:105-108.

27. Nielsen JB, Friberg L, Lou H, Lassen NA, Sam IL: **Immature pattern of brain activity in Rett syndrome.** *Arch Neurol* 1990, **47**:982-986.

28. D'Esposito M, Quaderi NA, Ciccodicola A, Bruni P, Esposito T, D'Urso M, Brown SD: **Isolation, physical mapping, and northern analysis of the X-linked human gene encoding methyl CpG-binding protein, *MECP2*.** *Mamm Genome* 1996, **7**:533-555.

29. Vilain A, Apiou F, Vogt N, Dutrillaux B, Malfoy B: **Assignment of the gene for methyl-CpG-binding protein 2 (*MECP2*) to human**

chromosome band Xq28 by in situ hybridization. *Cytogenet Cell Genet* 1996, **74**:293-294.

30. Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A: **Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA.** *Cell* 1992, **69**:905-914.

31. Tate P, Skarnes W, Bird A: **The methyl-CpG-binding protein MeCP2 is essential for embryonic development in the mouse.** *Nat Genet* 1996, **12**:205-208.

32. Nan X, Campoy FJ, Bird A: **MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin.** *Cell* 1997, **88**:471-481.

33. •• Coy JF, Sedlacek Z, Bachner D, Delius H, Poustka A: **A complex pattern of evolutionary conservation and alternative polyadenylation within the long 3′-untranslated region of the methyl-CpG-binding protein 2 gene (MeCP2) suggests a regulatory role in gene expression.** *Hum Mol Genet* 1999, **8**:1253-1262.

These authors describe extensive cross-species conservation in the 8.5 kb-long 3′UTR of MeCP2 and show that there is tissue-specific regulation of various transcripts. The existence of a 10.1 kb isoform that is highly enriched in the developing brain is interesting in view of the central nervous system phenotype of RTT.

34. Bird AP, Wolffe AP: **Methylation-induced repression — belts, braces and chromatin.** *Cell* 1999, **99**:451-454.

35. •• Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger Landsberger N, Strouboulis J, Wolffe AP: **Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription.** *Nat Genet* 1998, **19**:187-191.

See annotation [36••].

36. •• Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A: **Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex.** *Nature* 1998a, **393**:386-389.

Both studies [35••,36••] demonstrated *in vitro* and in cell culture experiments that after binding to methylated CpG dinucleotides, Mecp2 can recruit histone deacetylase to a transcriptional repressor complex and silence target genes. These studies prove, for the first time, that MBD-containing proteins such as Mecp2 are able to function as a molecular link between DNA methylation of promoter regions and transcriptional silencing.

37. Ng HH, Bird A: **DNA methylation and chromatin modification.** *Curr Opin Genet Dev* 1999, **9**:158-163.

38. Ashraf SI, Ip YT: **Transcriptional control: repression by local chromatin modification.** *Curr Biol* 1998, **8**:R683-R686.

39. Cooper DN, Krawczak M: *Human Gene Mutation.* Oxford: BIOS Scientific Publishers Limited; 1993.

40. Zoghbi HY, Percy AK, Schultz RJ, Fill C: **Patterns of X chromosome inactivation in the Rett syndrome.** *Brain Dev* 1990, **12**:131-135.

41. Webb T, Watkiss E, Woods CG: **Neither uniparental disomy nor skewed X-inactivation explains Rett syndrome.** *Clin Genet* 1993, **44**:236-240.

42. Anvret M, Wahlstrom J: **Rett syndrome: random X chromosome inactivation [Letter].** *Clin Genet* 1994, **45**:274-275.

43. Kormann-Bortolotto MH, Webb T: **Alterations in replication timing of X-chromosome bands in Rett syndrome.** *J Intellect Disabil Res* 1995, **39**:91-96.

44. Camus P, Abbadi N, Perrier MC, Chery M, Gilgenkrantz S: **X chromosome inactivation in 30 girls with Rett syndrome: analysis using the M27b probe.** *Hum Genet* 1996, **97**:247-250.

45. Schanen NC, Dahle EJ, Capozzoli F, Holm VA, Zoghbi HY, Francke U: **A new Rett syndrome family consistent with X-linked inheritance expands the X chromosome exclusion map.** *Am J Hum Genet* 1997, **61**:634-641.

46. Li E, Bestor TH, Jaenisch R: **Targeted mutation of the DNA methyltransferase gene results in embryonic lethality.** *Cell* 1992, **69**:915-926.

47. Hendrich B, Bird A: **Identification and characterization of a family of mammalian methyl-CpG binding proteins.** *Mol Cell Biol* 1998, **18**:6538-6547.