Role of Histone Methyltransferase G9a in CpG Methylation of the Prader-Willi Syndrome Imprinting Center*

Zhenghan Xin‡, Makoto Tachibana§, Michele Guggiarì¶, Edith Heard®, Yoichi Shinkai®, and Joseph Wagstaff**

From the Departments of ‡Biochemistry and Molecular Genetics and ¶Pediatrics, University of Virginia, Charlottesville, Virginia 22908-0733, §Department of Cell Biology, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan, and the ®Curie Institute, 75248 Paris, France

Imprinted genes in mammals are often located in clusters whose imprinting is subject to long range regulation by cis-acting sequences known as imprinting centers (ICs). The mechanisms by which these ICs exert their effects is unknown. The Prader-Willi syndrome IC (PWS-IC) on human chromosome 15 and mouse chromosome 7 regulates imprint gene expression bidirectionally within an ~2-megabase region and shows CpG methylation and histone H3 Lys-9 methylation in somatic cells specific for the maternal chromosome. Here we show that histone H3 Lys-9 methylation of the PWS-IC is reduced in mouse embryonic stem (ES) cells lacking the G9a histone H3 Lys-9/Lys-27 methyltransferase and that maintenance of CpG methylation of the PWS-IC in mouse ES cells requires the function of G9a. We show by RNA fluorescence in situ hybridization (FISH) that expression of Snrpn, an imprinted gene regulated by the PWS-IC, is biallelic in G9a−/− ES cells, indicating loss of imprinting. By contrast, Dnmt1−/− ES cells lack CpG methylation of the PWS-IC but have normal levels of H3 Lys-9 methylation of the PWS-IC and show normal monallelic Snrpn expression. Our results demonstrate a role for histone methylation in the maintenance of parent-specific CpG methylation of imprinting regulatory regions and suggest a possible role of histone methylation in establishment of these CpG methylation patterns.

Although the number of mammalian genes identified as showing parent-of-origin-specific expression patterns is growing, the mechanisms leading to their imprinted expression patterns are still poorly understood (1). For many imprinted genes, there is a correlation between parent-specific CpG methylation of 5′ regulatory regions and parent-specific expression. Targeted inactivation of the Dnmt1 DNA methyltransferase gene leads to disruption of imprinted expression for many imprinted genes (2). Imprinted genes are often located in clusters containing both maternally expressed and paternally expressed genes whose imprinting is subject to long range regulation by cis-acting sequences known as imprinting centers (ICs) (3–5).

One of the best characterized imprinting centers is the Prader-Willi syndrome (PWS) IC, which is required for establishment and maintenance of the paternal pattern of gene expression and CpG methylation in the Prader-Willi syndrome/Angelman syndrome (PWS/AS) region of human chromosome 15 and mouse chromosome 7 (3, 6, 7). The PWS-IC in human and mouse contains the promoter region of the imprinted Snrpn gene, and in both species, somatic cells show CpG methylation specific for the maternal copy of the PWS-IC. In mouse, this CpG methylation of the maternal PWS-IC occurs during oogenesis, and this methylation has been hypothesized to be the gametic imprint for the PWS/AS region in mouse (8). However, recent studies have shown that the PWS-IC is completely unmethylated in human oocytes (9); therefore, maternal-specific CpG methylation of this region must occur after fertilization, and some other epigenetic mark must constitute the gametic imprint.

We have shown previously in human somatic cells that histone H3 is modified by Lys-9 methylation on the maternal copy of the PWS-IC and that H3 is Lys-4-methylated on the paternal copy of the PWS-IC (10). We have proposed histone H3 methylation on Lys-9 in the PWS-IC as a candidate gametic imprint for the human PWS/AS region, with CpG methylation of the IC and of other promoter regions occurring as a consequence of the primary histone-based imprint. Recent studies by Tamara and Selker in Neurospora (11) and by Jackson et al. in Arabidopsis (12) have demonstrated dependence of cytosine methylation on histone H3 Lys-9 methyltransferases. In this study, we examine the hypothesis that CpG methylation of the mouse PWS-IC requires the histone H3 Lys-9/Lys-27 methyltransferase G9a.

EXPERIMENTAL PROCEDURES

Cell Lines—Undifferentiated G9a +/+; −/−, and −/− with G9a transgene ES cell lines (13), as well as a Dnmt1−/− ES cell line (14), were maintained in ES cell medium containing 10% fetal calf serum and 1000 units/ml ESGRO (Chemicon).

Chromatin Immunoprecipitation (ChIP)—Chromatin was prepared from ES cells as described (15) and was sonicated to an average size of ~0.5 kb. Spleens from F1 mice (C57Bl/6J x CAST/Ei) and CAST/Ei x C57Bl/6J were dissociated in Hanks’ balanced salt solution buffer and cross-linked with 1% formaldehyde for 10 min at room temperature. The fixed cells were collected by centrifugation at 700 × g for 10 min, resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-
carried out by PCR in the presence of bromide staining. Quantitative analysis of ChIP DNA (see Fig. 2) was

calculated as ([PWS-IC product from /-]/[D13Mit55 product from /-]) / ([PWS-IC product from /+]/[D13Mit55 product from /+]).

Bisulfite Genomic Sequencing—Bisulfite treatment of genomic DNA was carried out as described by Clark et al. (16). Bisulfite-modified DNA was amplified by nested PCR with the following primers: Snrpn 5' region (SNB1, 5'-GTGGATTTGTTAATTTGTGGAG-3' + SNB2, 5'-TTTCTACTTTAATAACAGCACCC-3'), and SNB3N, 5'-ATTATTCTTGAATGTAATG-3' + SNB4, 5'-ATCCCAACACCCCTACACTAA-3'). PCR products were cloned into a PCR-TOPO vector (Invitrogen) and sequenced.

RNA FISH—RNA FISH was performed as described (17). CITB mouse BAC 289D17 (clone obtained from Research Genetics) was used to detect Snrpn RNA; a clone 510 was used to detect Xist RNA.

RESULTS

To determine whether parent-specific H3 Lys-9 methylation at the PWS-IC is present in somatic cells of the mouse, we studied a single base pair variation between C57Bl/6J and Mus musculus castaneus (Cast-Ei) mice, located 104 bp 5' to the major site of Snrpn transcription initiation. Somatic tissues from F1 hybrids between C57Bl/6J and Cast-Ei were subjected to ChIP with antibody to methyl Lys-9 H3; sequencing of genomic DNA from reciprocal hybrids showed equal peaks corresponding to each of the parental alleles, but sequencing of PCR-amplified ChIP DNA showed only the maternal allele from each of the crosses (Fig. 1). Therefore, the maternal-specific association of methyl Lys-9 H3 with the PWS-IC region is conserved between human and mouse.

To determine which histone methyltransferase(s) is required for H3 Lys-9 methylation of the PWS-IC region in mouse, we examined ES cells homozygous for a targeted deletion of the gene encoding the G9a histone H3 Lys-9/Lys-27 methyltransferase (18). Loss of G9a function causes loss of H3 Lys-9 methylation primarily in euchromatic regions, rather than in regions of centromeric heterochromatin, and mice homozygous for the G9a deletion die in midgestation (13). ES cells homozygous for the G9a deletion are viable (13), and two independent

Fig. 1. Maternal-specific association of methyl Lys-9 H3 with the PWS-IC. Genomic DNA from F1 offspring of reciprocal crosses between C57Bl/6J and M. musculus castaneus-Ei (Cast-Ei) shows equal peaks at the variant site 104 bp 5' to the major start site of Snrpn transcription (arrow). DNA obtained by ChIP of spleen cells from the F1 mice with antibody specific for methyl Lys-9 H3 contains exclusively the maternal allele from both crosses. In the sequence, N indicates two coincident peaks.

Fig. 2. Association of PWS-IC with methylated H3 in G9a +/- and +/- ES cell lines. Lane 1, G9a +/-; lanes 2 and 3, G9a +/-; lane 4, G9a +/- with G9a transgene. a, analysis of DNA obtained by ChIP with antibody to methyl Lys-9 H3. Upper panel, PCR analysis of PWS-IC showing decreased PCR product visualized by ethidium bromide staining in G9a +/-; lane 2, PCR analysis of control locus D13Mit 55. b, analysis of DNA obtained by ChIP with antibody to methyl Lys-9 H3 with PCR products visualized by incorporation of [32P]dCTP and autoradiography. The ratio of PWS-IC PCR product to D13Mit55 product for each G9a +/- ES cell line was normalized to the ratio for the G9a +/+ cell line. c, analysis of DNA obtained by ChIP with antibody to methyl Lys-4 H3. Upper panel, PCR analysis of PWS-IC; lower panel, PCR analysis of control locus D13Mit55.

HCl, pH 8.0), and then sonicated to produce chromatin of an average size of ~0.5 kb. Chromatin was immunoprecipitated as described (15) with rabbit polyclonal antibody to dimethyl Lys-9 H3 or with rabbit polyclonal antibody to dimethyl Lys-4 H3. DNA recovered from the immunoprecipitated material was amplified by PCR with the following primers: PWS-IC (Snrpn 5' region, Msnp1, 5'-AGACGCCTCAATTCGCCAG-3' and Msnp2, 5'-TACTGTCGCACATTGCT-3'), and D13Mit55 (D13Mit-F, 5'-TCAATATACGTCATGCAGTGTT-3' and D13Mit-R, 5'-GGTCTTCTCCCAACCACT-3'). Amounts of ChIP DNA per reaction were normalized to give equal amounts of product with D13Mit55 control primers, which amplify a locus near the centromere of mouse chromosome 13; PCR yield with D13Mit55 primers on CHIP DNA is independent of G9a genotype for ChIP with antibodies to methyl Lys-9 H3 and methyl Lys-4 H3 (data not shown). Annealing temperature was 59 °C for Msnp1/Msnp2 and 55 °C for D13Mit-F/ D13Mit-R. Products in Fig. 2, a and c, were visualized by ethidium bromide staining. Quantitative analysis of ChIP DNA (see Fig. 2b) was carried out by PCR in the presence of [32P]dCTP for 20 cycles, where product yield is a linear function of cycle number for both primer sets. PCR products were separated on 12% nondenaturing polyacrylamide gels and visualized by autoradiography, and films were scanned with an Amersham Biosciences densitometer and analyzed with ImageQuant software. ChIP DNA PCR ratios for the two G9a +/- ES cell lines were

Fig. 3. G9a +/- ES cells lack CpG methylation of the PWS-IC. Bisulfite genomic sequencing of the PWS-IC region in G9a +/- and +/- ES cells shows dependence of CpG methylation on G9a function. Each row of circles connected by a horizontal line represents one PCR product from bisulfite-treated DNA that has been cloned and sequenced. The amplified region (GenBank™ accession number AC026878, nucleotides 135219–135499) contains 16 CpG dinucleotides, each represented by a circle. Closed circles indicate methylated cytosine residues (not converted to uracil by bisulfite treatment), and open circles indicate unmethylated cytosine residues that have been converted to uracil by bisulfite treatment. (Snrpn transcription starts at nucleotide 135416.) Results are shown for the parental G9a +/- ES cell line and three G9a +/- ES cell lines (independent homozygous derivatives of a G9a +/- ES cell line).
Signal, Snrpn. FISH analysis of G9a/H11002

14998

G9a. Role in Imprinting-associated CpG Methylation

G9a role in imprinting-associated CpG methylation

G9a and Snrpn expression was performed in male ES cells. Red signal, Snrpn; green signal, Xist. G9a +/+ (a) and +/− (b) ES cells show either a single strong Snrpn signal or one strong Snrpn signal and one very weak signal per nucleus. G9a −/− ES cells (c and d) show two strong Snrpn signals in >80% of cells. All cells have a single copy of Xist, the X-inactive specific transcript, on the X-chromosome. (c) Situated female ES cell line; (d) ES cell line. Ectopic expression of G9a in male ES cells. Greater than 80% of G9a +/+ or +/− ES cells showed either a single Snrpn signal or one strong and one very weak G9a signal per nucleus (Fig. 4, a and b); by contrast, >80% of G9a −/− ES cells from two lines showed two strong Snrpn hybridization signals, indicating biallelic expression (Fig. 4, c and d). The observation of one strong and one very weak Snrpn RNA FISH signal in G9a wild-type nuclei suggests that Snrpn imprinting in ES cells is slightly leaky, unlike the situation in somatic tissues; results consistent with this observation have been reported by Szabo and Mann (19). G9a −/− ES cells with a G9a transgene also showed two Snrpn signals in >80% of cells, although the intensity of the two signals within each nucleus tended to be less equal than in the G9a −/− ES cells (data not shown).

We also tested methylation of CpG sites in the PWS-IC region in embryonic day 9.5 G9a +/+ and −/− embryos arising from heterozygote intercrosses. We found no significant difference between the CpG methylation patterns in G9a +/+ and −/− embryos (Fig. 5).

To determine whether CpG methylation of the PWS-IC region is required for normal levels of H3 Lys-9 methylation, we performed bisulfite genomic sequencing and ChIP analysis of a Dnmt1 −/− ES cell line. Bisulfite genomic sequencing of 10 clones showed a complete absence of CpG methylation of the PWS-IC region (data not shown). ChIP analysis showed that the association of the PWS-IC region with methyl Lys-9 H3 was unaffected by targeted inactivation of the Dnmt1 maintenance DNA methyltransferase (Fig. 6). RNA FISH analysis of the Dnmt1 −/− ES cells showed that >80% of cells contained either a single Snrpn signal or one strong and one very weak G9a signal per nucleus, as in wild-type ES cells (Fig. 7).

Discussion

Our data show that the PWS-IC is H3 Lys-9-methylated specifically on the maternal allele, that H3 Lys-9 methylation

Fig. 4. G9a −/− ES cells show loss of imprinting of Snrpn. RNA FISH analysis of Snrpn expression was performed in male ES cells. Red signal, Snrpn; green signal, Xist. G9a +/+ (a) and +/− (b) ES cells show either a single strong Snrpn signal or one strong Snrpn signal and one very weak signal per nucleus. G9a −/− ES cells (c and d) show two strong Snrpn signals in >80% of cells. All cells have a single copy of Xist that is either unreplicated (a, c, and d) or replicated (b).

Fig. 5. Bisulfite genomic sequencing of the PWS-IC region from G9a +/+ and −/− embryonic day 9.5 embryos. Analysis was carried out in DNA from one +/+ embryo and two −/− embryos as described in the legend for Fig. 3 and shows no significant effect of the G9a genotype on CpG methylation.

G9a −/− ES cell lines both showed reduction of H3 Lys-9 methylation at the PWS-IC to ∼30% of G9a +/+ levels (Fig. 2). Total H3 Lys-9 methylation of the PWS-IC in G9a −/− ES cells with a G9a transgene was similar to that in G9a +/+ ES cells (Fig. 2). G9a inactivation did not have any significant effect on the level of H3 Lys-4 methylation in the PWS-IC (Fig. 2).

DNA from G9a +/+ and −/− ES cells was analyzed by bisulfite genomic sequencing (16) to determine the methylation status of CpG dinucleotides in the PWS-IC region. Seven out of ten clones from +/+ cells showed evidence of methylation at all or almost all CpG sites (Fig. 3), and five out of nine clones from −/− cells showed methylation at all or almost all sites (data not shown); by contrast, 32 clones from 3 G9a −/− cell lines showed no evidence of methylation at any CpG site (Fig. 3). G9a −/− ES cells with a G9a transgene showed no evidence of methylation at any CpG site (data not shown).

We examined the role of G9a in control of imprinted Snrpn expression by RNA FISH analysis of ES cell lines. Simultaneous Xist RNA FISH was performed as a control as this transcript can normally be detected in almost 100% of undifferentiated male ES cells. Greater than 80% of G9a +/+ or +/− ES cells showed either a single Snrpn signal or one strong and one very weak Snrpn signal per nucleus, as in wild-type ES cells (Fig. 7).

Discussion

Our data show that the PWS-IC is H3 Lys-9-methylated specifically on the maternal allele, that H3 Lys-9 methylation

Fig. 6. Association of PWS-IC with Lys-9-methylated H3 in Dnmt1 +/+ and −/− ES cell lines. DNA obtained by ChIP with antibody to methyl Lys-9 H3 from Dnmt1 +/+ and −/− ES cells was analyzed by PCR for the PWS-IC (top panel) and for the control locus D13Mit55 (lower panel).
of the PWS-IC region is reduced in G9a−/− ES cells, and that maternal-specific CpG methylation of the PWS-IC is lost in G9a−/− ES cells. Previous authors have suggested that CpG methylation of the maternal copies of some imprinting centers, established in the female germline, serves as the gametic imprint for coordinately regulated clusters of imprinted genes such as those in the PWS/AS region and in the Beckwith-Wiedemann syndrome (BWS) region (1). Although mouse data showing CpG methylation of the PWS-IC and the BWS-IC2 in oocytes but not in sperm are consistent with this hypothesis (4, 8), the recent demonstration that the PWS-IC is completely unmethylated on CpG dinucleotides in human oocytes (9) implies that parent-specific CpG methylation differences at the human PWS-IC occur after fertilization and that CpG methylation of the human PWS-IC cannot be the gametic imprint for the human AS/PWS region.

Tamaru and Selker (11) have shown that CpG methylation in Neurospora requires the Dim-5 H3 Lys-9 methyltransferase, and Jackson et al. (12) have demonstrated that CnpNP methyltransferase in Arabidopsis is dependent on the H3 Lys-9 methyltransferase KYP. We have demonstrated that maintenance of CpG methylation at the PWS-IC in mouse ES cells is also dependent on an H3 Lys-9/Lys-27 methyltransferase, G9a. The mechanisms leading to dependence of cytosine methylation on histone H3 Lys-9 methyltransferases in these three systems have not yet been elucidated. G9a+/− and −/− ES cells show no differences in DNA methyltransferase activities or in expression levels of Dnmt1, Dnmt3a, or Dnmt3b. The level of H3 Lys-9 methylation of the PWS-IC in G9a−/− ES cells is reduced to ~30% of that observed in G9a+/+ ES cells. The residual H3 Lys-9 methylation in G9a−/− ES cells must be maintained by a histone methyltransferase other than G9a; either the methyltransferase or the pattern of Lys-9 methylation catalyzed by that methyltransferase must be unable to participate in maintaining CpG methylation of the PWS-IC.

We have shown that restoration of G9a function in G9a−/− ES cells with a transgene leads to near normal levels of H3 Lys-9 methylation of the PWS-IC but does not restore CpG methylation or monoallelic Snrpn expression. This result suggests that although G9a is required for maintenance of CpG methylation at the PWS-IC in ES cells, its presence is not sufficient for de novo CpG methylation of the PWS-IC, which must require molecules that are not present in ES cells. Biniszkiewicz et al. (20) have shown that overexpression of Dnmt1 in Dnmt1−/− ES cells does not lead to CpG methylation of the Snrpn promoter, and they have suggested that de novo CpG methylation of this region can occur only during gametogenesis. G9a transgene expression in G9a−/− ES cells is also clearly not a sufficient condition for monoallelic expression of Snrpn.

Although G9a−/− ES cells show complete loss of CpG methylation at the PWS-IC, we have seen no effect of the G9a mutation on CpG methylation of these regulatory regions in embryonic day 9.5 embryos. The reason for this difference is unclear. In both cases, the observed cytosine methylation represents maintenance, rather than de novo, methylation. Our results may indicate the existence of histone methylation-independent mechanisms for maintenance of CpG methylation at imprinting centers in embryos but not in ES cells; alternatively, other H3 methyltransferases may compensate for loss of G9a function in embryos but not in ES cells.

We have seen no effect of targeted mutation of the Dnmt1 maintenance cytosine methyltransferase on H3 Lys-9 methylation of the PWS-IC. In the ES cell system, dependence of CpG methylation of the PWS-IC on the G9a H3 Lys-9/Lys-27 methyltransferase does not appear to be accompanied by a reciprocal dependence of H3 Lys-9 methylation on CpG methylation. RNA FISH analysis of these Dnmt1−/− ES cells showed normal monoallelic expression of Snrpn, indicating that CpG methylation of the PWS-IC is not required for monoallelic expression of Snrpn in ES cells. Previous studies of imprinted gene expression in undifferentiated Dnmt1−/− ES cells have not distinguished monoallelic from biallelic expression; however, Tucker et al. (21) showed that the level of expression of Igf2r, which is normally maternally expressed, is the same in Dnmt1+/- and −/− ES cells. Our results suggest that, in undifferentiated ES cells, histone H3 Lys-9 methylation is sufficient and CpG methylation is not required for maintenance of Snrpn monoallelic expression. We hypothesize that the pattern of H3 Lys-9 methylation of the PWS-IC in G9a−/− ES cells with a G9a transgene is not the normal pattern established during gametogenesis, both in terms of allele specificity and distribution on nucleosomes, and that this qualitative abnormality of H3 Lys-9 methylation accounts for the biallelic expression of Snrpn in these cells. This hypothesis is difficult to test because the parental ES cell line used in these studies contains very few heterozygous polymorphic sites so that it is not possible to test for allele-specific association of modified histones with the PWS-IC and because it is very difficult to assess the sensitivity of ChiP to the density of histone modifications.

Previously we have proposed, based on our observations of maternal-specific association of methyl Lys-9 H3 with the PWS-IC and on the lack of CpG methylation of the PWS-IC in human oocytes, that H3 methylation may be the gametic imprint for the maternal allele of the human AS/PWS region and may trigger CpG methylation of the PWS-IC and parent-specific expression throughout the AS/PWS region after fertilization (10). The data presented here, showing dependence of CpG methylation at the mouse PWS-IC on the G9a histone methyltransferase, are completely consistent with this hypothesis. Most putative germline imprints involve repression on the maternal alleles (22), and a histone-modification-based gametic imprint can only be carried on the maternal allele because histones are replaced by protamines during spermatogenesis. It is clear that the PWS-IC is CpG methylated in mouse oocytes, unlike the situation in human oocytes. We hypothesize that this difference does not reflect a fundamental difference between mice and human in the imprinting process, but rather that the difference is in the timing of histone methylation-dependent CpG methylation: prefertilization in the mouse, postfertilization in human. Our data do not address the question of the role of G9a and histone methylation in the establishment of imprints in the germline; an answer to this question will require conditional knockouts of G9a and other histone methyltransferases limited to the germline.

Acknowledgments—We thank C. David Allia for antibodies to methyl Lys-9 H3 and methyl Lys-4 H3 and for helpful comments. We thank En Li for the Dnmt1−/− ES cell line. We also thank Jason Lau for unpublished data regarding the D13Mit55 locus.

REFERENCES

1. Ferguson-Smith, A. C., and Surani, M. A. (2001) Science 293, 1086–1089
2. Li, E., Beard, C., and Jaenisch, R. (1998) Nature 398, 362–365
3. Nicholls, R. D., Saitoh, S., and Horsthemke, B. (1998) Trends Genet. 14, 194–200
4. Engemann, S., Strodicke, M., Paulsen, M., Franck, O., Reinhardt, R., Lane, N., Rei, W., and Walter, J. (2000) Hum. Mol. Genet. 9, 2691–2706
5. Zwart, R., Sleutels, F., Wutz, A., Schinkel, A. H., and Beaudet, A. L. (2001) Genes Dev. 15, 2361–2386
6. Yang, T., Adamson, T. E., Renwick, J. L., Leff, S., Wierwick, R., Francke, U., Jenkins, N. A., Copeland, N. G., and Brannan, C. I. (1998) Nat Genet. 19, 25–31
7. Bressler, J., Tsai, T.-F., Wu, M.-Y., Tsai, S.-F., Ramirez, M. A., Armstrong, D., and Beaudet, A. L. (2001) Nat Genet. 28, 232–240
8. Shemer, R., Birger, Y., Riggs, A., and Razin, A. (1997) Proc. Natl. Acad. Sci.

2 M. Tachibana and Y. Shinkai, unpublished data.
15000

G9a Role in Imprinting-associated CpG Methylation

U. S. A. 94, 10267–10272
9. El-Maarri, O., Buiting, K., Peery, E., Krause, P., Balaban, B., Wagner, K., Urman, B., Heyd, J., Lich, C., Brannan, C., Walter, J., and Horsthemke, B. (2001) Nat. Genet. 27, 341–344
10. Xin, Z., Allis, C. D., and Wajstaf, J. (2001) Am. J. Hum. Genet. 69, 1389–1394
11. Tamaru, H., and Selker, E. U. (2001) Nature 414, 277–283
12. Jackson, J. P., Lindroth, A. M., Cao, X., and Jacobsen, S. E. (2002) Nature 416, 556–569
13. Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., Fukuda, M., Takeda, N., Niida, H., Kato, H., and Shinkai, Y. (2002) Genes Dev. 16, 1779–1791
14. Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1999) Cell 99, 247–257
15. Kuo, M.-H., and Allis, C. D. (1999) Methods (Orlando) 19, 425–433
16. Clark, S. J., Harrison, J., Paul, C. L., and Frommer, M. (1994) Nucleic Acids Res. 22, 2590–2597
17. Heard, E., Rougeulle, C., Arnaud, D., Avner, P., Allis, C. D., and Spector, D. L. (2001) Cell 107, 727–738
18. Tachibana, M., Sugimoto, K., Fukushima, T., and Shinkai, Y. (2001) J. Biol. Chem. 276, 23089–23101
19. Szabo, P. E., and Mann, J. R. (1994) Development 120, 1651–1660
20. Binzskiewicz, D., Gribnau, J., Ramsahoye, R., Gaudet, F., Eggan, K., Humphrys, D., Mastrangelo, M. A., Jun, Z., Walter, J., and Jaenisch, R. (2002) Mol. Cell. Biol. 22, 2124–2135
21. Tucker, K., Beard, C., Daum, J., Jackson-Grusby, L., Laird, P., Lei, H., Li, E., and Jaenisch, R. (1996) Genes Dev. 10, 1008–1020
22. Reik, W., and Walter, J. (2001) Nat. Genet. 27, 255–256
