A large fraction of the animal genome is maintained in a transcriptionally repressed state throughout development. By generating viable Dmnt1−/− mouse cells we have been able to study the effect of DNA methylation on both gene expression and chromatin structure. Our results confirm that the underlying methylation pattern has a profound effect on histone acetylation and is the major effect of me-H3(K4) in the animal genome. We demonstrate that many methylated genes are subject to additional repression mechanisms that also impact on histone acetylation, and the data suggest that late replication timing may play an important role in this process.

The transcription of many genes in the animal genome is stably repressed. These include tissue-specific genes that are constitutively silenced in almost all cell types and only activated in their tissue of expression, as well as embryonic genes that are turned off during early development and then remain repressed in most cell types. These observations strongly suggest the existence of basic mechanisms capable of maintaining repression through the repeated cell divisions that take place during development.

One of the main mechanisms for maintaining stable gene repression is DNA methylation. The animal genome is characterized by a distinct bimodal pattern of methylation in which most gene regions are methylated whereas CpG islands mainly located in housekeeping genes are constitutively unmethylated (1). This profile is established at the time of implantation by a wave of de novo methylation (2) and is then maintained basically intact throughout development, although some genes undergo selective demethylation in their tissue of expression. Because of this pattern it has been proposed that DNA methylation may play a role in the global repression of background transcription from genomic sequences (3). This idea is strongly supported by experiments in tissue culture and in vivo that show that exogenously introduced methylated DNA is repressed and packaged in a closed chromatin conformation (4) through a process that is probably mediated by methyl-binding proteins (5).

Although these studies show that methylation can inhibit gene expression by itself, they do not prove that all methylated genes in the genome are stably repressed exclusively by this modification, and, indeed, other well-characterized mechanisms may also take part in this process. Many genes, for example, are stably silenced by means of trans-acting repressor factors that are constitutively available to bind specific sequence motifs near gene promoters. These proteins apparently operate by directly influencing the transcription machinery or by affecting local chromatin structure through nucleosome positioning or histone modification (6).

Another epigenetic marker that may affect gene repression is DNA replication timing (7). The entire genome appears to be subdivided into chromosome bands, each of which is set up to replicate in a specific time window during S phase. Housekeeping genes are clustered (8) in regions that constitutively replicate early, whereas many tissue-specific genes replicate late in most cell types but are developmentally regulated to become early replicating in their tissue of expression. Recent microinjection studies suggest that the time of replication itself could influence gene activity and structure (9). These experiments showed that DNA replicating in early S phase gets automatically repackaged with acetylated histones, whereas the regions that replicate late in S phase assemble nucleosomes containing deacetylated histones. Because this process is repeated in every cell cycle, late replication timing could represent another potential mechanism for maintaining gene repression.

To understand the role of DNA methylation within the framework of additional repression mechanisms, we have generated DNA methyltransferase-minus (Dmnt1−) primary fibroblasts that have almost all CpG sites unmethylated. Using this system we surveyed a large number of individual genes in the mouse genome, analyzing their DNA methylation state, expression pattern, histone modification profile, and replication timing status. This has enabled us to unravel how DNA methylation works together with other basic repression mechanisms to generate distinct paradigms of gene silencing.
EXPERIMENTAL PROCEDURES

Cells—The P cell line was established by culturing mouse embryo fibroblast cells from 9.5-day p53−/− embryos (10). Primary embryonic fibroblasts (wild-type) were grown from 13.5-day mouse embryos. The PM cell line (p53−/− Dnmt1−/−) was obtained from 9.5-day embryos generated by crosses between p53−/− Dnmt1−/− animals carrying the n allele (11) and grown on gelatin-coated plates in Dulbecco’s modified Eagle’s medium supplemented with l-glutamine, non-essential amino acids, sodium pyruvate, β-mercapto-ethanol, and 15% fetal calf serum. Normal tissues were dissected from C57Bl mice. Treatment with the histone deacetylase inhibitor trichostatin A (TSA)4 was performed at 20 ng/ml, a concentration that does not interfere with the cell cycle in fibroblast cells (data not shown). Under these conditions, the effects of forced deacetylation appear to be within the physiological range and are completely reversible once the drug is removed (see below).

RT-PCR—Total RNA (250 ng) was prepared with the TriPure isolation reagent (Roche Applied Science) and converted to cDNA using the M-MLV reverse transcriptase (Promega) and random hexanucleotide pd(N)6 primers (GE Healthcare) in a reaction volume of 20 μl under conditions recommended by the manufacturer. cDNA was used for PCR reactions (95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min) in the presence of [α-32P]dCTP (Amersham Biosciences) and different primer pairs (sequences available upon request) designed to span intron-exon junctions in order to distinguish between cDNA and genomic DNA. RT-PCR fragments were separated on 5% polyacrylamide gels and exposed for autoradiography. Relative steady state RNA levels for each gene sequence were determined by carrying out serial dilutions of cDNA and comparing the amount of PCR product to that obtained for Pck1 (set at 1) from undiluted cDNA of PM cells induced with TSA using the same number of cycles. The results were quantitated by phosphorimaging analysis. It should be noted that repeat assays indicate that this method has a coefficient of variation of ~20%. In addition, real-time PCR analysis of the same samples yielded very similar results (~25%). Microarray analysis was carried out on the same RNA samples using Affymetrix (MOE 430A) chips and standard data computational techniques (GenBankTM accession number GSE3534).

Chromatin Immunoprecipitation—Sonicated cross-linked chromatin or mono- and multimeric nucleosome fractions were prepared and chromatin immunoprecipitation analysis performed using anti-Ac-H4, Ac-H3(K9, K14) and 2,3me-H3(K4) (Upstate Biotechnology) to separate bound from input fractions (12). Because we usually precipitated less than 1% of the nucleosomes in this procedure, real-time or semiquantitative PCR of the bound fraction was compared with 1/100 dilutions of the input DNA. Semi-quantitative PCR was carried out on two concentrations (1 or 3 μl) of sample in the presence of [α-32P]dCTP (Amersham Biosciences) using promoter-specific primer pairs (sequences available upon request). Enrichment (Bound/Input) was calculated from phosphorimaging analysis and normalized to take into consideration the relative amount of DNA in the bound and input fractions. For this purpose, Pck1 served as a negative control. It should be noted that in order to avoid misinterpretation all of the genes used for chromatin immunoprecipitation analysis were carefully selected to ensure that these are indeed unique gene sequences whose expression is derived exclusively from a single promoter region.

DNA Methylation and CpG Island Analysis—Gene promoter methylation was determined by the technique of methylated DNA immunoprecipitation. This method provides a linear assessment of the degree of methylation (13, 14). Genes found methylated by this assay that were retested by bisulfite analysis showed methylation levels >70%. DNA from mono- and multimeric nucleosome fractions of P or PM cells was purified by phenol extractions and ethanol precipitation, mixed with human mononucleosome DNA, denatured, and immunoprecipitated by an anti-5-methylcytidine antibody (15). Bound and input fractions were subjected to semi-quantitative PCR from specific gene regions using two concentrations (1 or 3 μl) of DNA in the presence of [α-32P]dCTP (Amersham Biosciences). Because we usually precipitated less than 1% of the DNA, PCR of the bound fraction was compared with 1/100 dilutions of the input DNA (see supplemental Fig. S1A). Calibration was carried out by comparing a known unmethylated (APRT) to methylated (CRYAA) promoter fragment from the human carrier DNA. A gene was considered to have a CpG island promoter if it contained a 200-bp stretch of DNA with a C+G content of 50% and an observed CpG/expected CpG in excess of 0.6 (16) directly upstream to the transcription start site. Promoters of genes on the microarray were identified using Promoter (biowulf. bu.edu/zlab/promoser). Methylation of some genes was also tested by restriction analysis (see supplemental Fig. S1B).

Microarray Analysis—RNA from P and PM cells with or without treatment with TSA (20 ng/ml) for 24 and 48 h was prepared and used to make fluorescent-labeled DNA that was then hybridized to Affymetrix chips (MOE 430A) carrying 22,634 mouse cDNA segments, and the results were quantitated using standard protocols, taking advantage of internal gene controls. The levels of RNA (R) ranged from values of <1 to 6300. The data from 24 and 48 h of TSA treatment were averaged together before analysis. Because this method loses accuracy below a certain threshold, we set all of the expression levels below 10 to a floor value of 10. The microarray hybridization method (Fig. 2B) is less sensitive than calibrated RT-PCR analysis (Fig. 1C), and many genes measured by RT-PCR were actually below the threshold of detection in the microarray analysis. Furthermore, the changes observed in Dnmt1−/− cells or after TSA treatment, although detected for some of the genes, were clearly blunted in comparison to the RT-PCR results. The cluster analysis shown in Fig. 2 was carried out by calculating ln(R/Av) for all test samples of each gene and assigning colors to these values, ranging from green (low) to red (high) (Av = average R for the test samples).

RESULTS

Stable Dnmt1−/− Fibroblasts—To evaluate how DNA methylation affects gene repression, we developed a stable somatic cell line lacking Dnmt1 activity. This was accomplished by crossing

4 The abbreviations used are: TSA, trichostatin A; RT-PCR, reverse transcription PCR.
Repression by DNA Methylation

Dnmt1−/− with p53−/− mice (P) and culturing primary fibroblasts from 9.5-day p53−/− Dnmt1−/− embryos prior to their death, which would normally occur at about embryonic day 10.5. After a short lag, these mutant cells (PM) grow at normal exponential rates and do not undergo senescence even after 100 generations in culture (see “Experimental Procedures”). Because these cells have only a low level of maintenance methylation activity (11) and because fibroblasts, as opposed to embryonic stem cells, lack general de novo methylation ability, methyl groups are irreversibly lost in each replication cycle, and following a small number of divisions, the entire genome reaches a very low level of modification as compared with either wild-type or p53−/− fibroblasts (Fig. 1A). We were unable to culture primary fibroblasts from p53−/−/Dnmt1−/− embryos, suggesting that p53 may be essential for protecting the cell from massive demethylation.

Effect of Undermethylation and TSA on Gene Expression—Previous microarray studies identified gene sequences that are automatically induced in Dnmt1−/− cells, presumably because of forced undermethylation at their promoter (17). In contrast, many standard tissue-specific genes with methylated promoters did not seem to be affected by the lack of Dnmt1 in these analyses, giving the impression that DNA methylation may not play a role in their repression. In order to re-address this issue we selected nine candidate genes of this nature and subjected them to semi-quantitative RT-PCR analysis in both P and PM cells. Because methylation often operates in collaboration with histone deacetylation to repress gene expression (18), we also treated these same cells with the histone deacetylase inhibitor TSA (Fig. 1B). This allowed us to detect the effect of demethylation alone (untreated PM cells) as well as demethylation in combination with histone acetylation (TSA-treated PM cells).

These experiments substantiate the idea that there are at least two distinct classes of methylated genes. One category (group A) is automatically induced following the removal of DNA methylation. Although we only examined two specific genes (Slp1 and Rhox5), many other sequences of this nature have been picked up by previous microarray analysis (17). In addition, some large gene families, including endogenous viral sequences (19), Maspin (20) and Mage (21), appear to be regulated in a similar manner.

All of the other methylated genes we tested (group B) could not be activated by simple demethylation but underwent a dramatic induction when subjected to combined demethylation and forced acetylation (e.g. Hbb-b1 and Cryaa). Thus, although both group A and group B genes are affected by demethylation, it is clear that there is a basic difference in their regulation profiles, and the data suggest that in addition to DNA methylation, group B must be subject to other repression mechanisms. In keeping with this, serial semi-quantitative RT-PCR analysis of this small sample suggested that group B sequences may actually be expressed at levels about 3 orders of magnitude lower than any of the genes in group A (Fig. 1C).

Global Gene Expression Analysis—To confirm, in a more general manner, that methylated genes indeed fit into these two basic repression paradigms, we carried out expression microarray analysis on P and PM cells with or without TSA treatment. This survey, which included over 22,000 mouse gene segments, enabled us to identify many new non-CpG island genes with expression patterns similar to those of group A- or group B-type regulation (Fig. 2, A and B). A number of sample genes in each category were selected from the microarray data, tested for DNA methylation by methylated DNA immunoprecipitation (see “Experimental Procedures”).

In compiling the list of genes that undergo direct induction in Dnmt1−/− (PM) fibroblast cells, we noted that many of them are actually transcribed from CpG island promoters (see also Ref. 17) that are constitutively unmethylated. It is very likely that these were inhibited either through direct interaction with the Dnmt1 repression domain itself (22, 23) or by indirect effects caused by demethylation of other regulatory sites in the

5 H. Cedar, unpublished results.
Repression by DNA Methylation

A

Group A

Group B

B

C

Time after treatment (days)

| Name | CpG | Me | P | TSA | PM | PM+TSA |
|------|-----|----|---|-----|----|--------|
| Tif  | -   | +  | 10| 10  | 1400| 1200   |
| Matr | -   | +  | 10| 10  | 1400| 1500   |
| P91k3| +   | +  | 16| 16  | 410 | 420    |
| actT.14| + | +  |10|10  |1900|2200    |
| Tral | -   | +  | 10| 10  | 260 | 190    |
| KftT.7| - | +  |20| 10  | 300 | 465    |
| Atr  | -   | +  | 10| 10  | 19  | 46     |
| Myd  | -   | +  | 10| 10  | 19  | 46     |
| SykT | -   | +  | 10| 10  | 19  | 46     |
| Jbg2 | +   | +  | 10| 10  | 19  | 46     |
| StarPa | +   | +  |10|10 |10 | 30     |
| Par  | -   | +  | 10| 10  | 19  | 46     |
| Sol2 | -   | +  | 10| 10  | 19  | 46     |

WK_Apr | + | -  | 1500| 1500| 2700| 2800 |

FIGURE 2. Microarray analysis. Microarray analysis of RNA from P and PM cells with or without treatment with TSA was carried out as described under "Experimental Procedures." A, examples of non-CpG island promoter genes induced in PM cells (Group A) or following treatment of PM cells with TSA (Group B) by more than 2 S.D. from the mean are depicted in a cluster chart ("Experimental Procedures") in which levels vary from low (green) to high (red). Expression of group B genes was also analyzed 2 weeks (chase) following a 24-h TSA treatment (last column). B, the expression results for selected genes from this survey are shown in table form. These same genes were subsequently used for chromatin analysis (Fig. 3). C, RT-PCR results for selected group B genes in PM cells 0, 2, 9, and 13 days following a 24-h treatment with TSA.

Genome. Because our experiments are focused exclusively on the effect of DNA methylation in cis, genes of this type were not included.

Histone Modification Patterns—All of the basic repression mechanisms in animal cells appear to work, at least in part, by affecting local histone acetylation (24). Thus, in order to understand the expression patterns of methylated genes at the mechanistic level, we used chromatin immunoprecipitation to analyze histone modification profiles over the promoter sequences of select genes from groups A and B in both P and PM cells (Fig. 3, A and B and supplemental Fig. S2). In this assay, active unmethylated CpG island housekeeping genes such as Actb display a relatively open structure that is enriched for acetylated histones H3(Ac-H3) and H4(Ac-H4) and for histone H3 methylated on lysine 4 (Me-H3(K4)). In contrast, all of the methylated genes in our survey are depleted for Ac-H3, Ac-H4, and Me-H3(K4) (Fig. 3), and this is consistent with previous studies showing that the presence of DNA methylation can remodel local histone modification patterns (25).

In the case of group A genes, removal of methyl groups from the DNA, as occurs in PM cells, brings about a significant change in structure characterized by partial reacetylation of histones H3 and H4 and remethylation of H3(K4) (Fig. 3). These data suggest that for this category histone deacetylation at the promoter is brought about largely by underlying DNA methylation, probably through the action of methyl-binding proteins such as MeCP2 or MBD2 that can recruit histone deacetylases (5).

In contrast to these genes, removal of DNA methylation from group B promoters does not result in any appreciable histone reacetylation (Fig. 3). This finding provides support for the observation that transcription of these genes is not directly induced in PM cells and indicates that there must be additional mechanisms for bringing about deacetylation at these gene promoters. In contrast to acetylation, removal of DNA methylation almost always causes partial remethylation of H3(K4), suggesting that this histone modification is a direct reflection of the DNA methylation state. These experiments are summarized in Fig. 3B.

Experiments in yeast have suggested that histone acetylation states at specific loci can be maintained independently even without the continued presence of a basic mechanism for their establishment (26). To test this idea in our animal cell model, we treated P and PM cells with a pulse of TSA and then followed the RNA levels of several genes in group B over time. Even though these genes are induced as a result of TSA treatment and have obviously undergone de novo histone acetylation (see Ref. 27), they still become repressed again after removal of the drug (Fig. 2B). This behavior was also confirmed by microarray analysis (Fig. 2A). These results strongly suggest that even in the absence of methylation the deacetylated state (as revealed by response to TSA) is not self-perpetuating and must be continually regenerated by additional underlying repression mechanisms.

Replication Timing—To identify additional mechanisms that may contribute to the repression of group B genes, we assessed the replication timing profiles of all the genes in our survey. This was carried out using PCR analysis of bromodeoxyuridine-labeled DNA from S phase-fractionated cells (Fig. 4), a method that allows multiple assays on each sample and produces a good snapshot of replication timing in the genome (28). As a first step, we assayed the replication profile of ten unmethylated CpG island genes, and all of them were found to replicate in early S phase regardless of their expression level (data not shown). Genes whose repression is dependent exclusively on DNA methylation (group A) also appear to replicate uniformly in early S phase. In contrast, ~50% of the group B genes in our
survey replicate late in S phase (Fig. 3, A and B). The fact that late replication is only observed in this group is highly significant (p < 0.001) and strongly suggests that this epigenetic feature may represent one of the mechanisms employed for mediating gene repression in combination with DNA methylation.

DISCUSSION

Basic Repression Mechanisms—A large percentage of the genome is programmed to be repressed in almost every cell of the organism (6). This requires molecular mechanisms that can preserve the inactive state by regenerating a repressive chromatin structure following each round of DNA replication. DNA methylation is one of the fundamental mechanisms known to be involved in this maintenance process. To study the role of DNA methylation within the context of other repression mechanisms that operate in the nucleus, we developed a Dnmt1−/− cell line in which almost the entire genome is in an unmethylated state. Our studies show that there are two distinct categories of methylated genes. Although many of them are constitutively repressed mainly by the presence of methyl groups at their promoter, a large number of methylated genes are kept in their inactive state through the involvement of multiple repression modalities that may work in a layered manner.

By analyzing the histone modification pattern of methylated genes, we have been able to gain some insight into the molecular logic involved in the organization of genome-wide repression. All of the methylated genes in our survey were found to be packaged with deacetylated histones at their promoter, and in every case, reacetylation appears to be a necessary precondition for transcriptional reactivation. For genes in group A, DNA methylation constitutes the principal mechanism for bringing about local deacetylation, and once the methyl groups are forcibly removed the DNA becomes packaged in a relatively open, acetylated structure. Other methylated genes (e.g. group B), however, are probably subject to additional molecular mechanisms that also operate, at least in part, through histone deacetylation (Fig. 5). For this reason, the removal of methyl groups from these genes is not sufficient for bringing about histone reacetylation, and they remain transcriptionally repressed.

Gene repression paradigms in animal cells appear to be set up early in development and are then stably maintained through each cycle of DNA replication and cell division. It is important to note that the histone acetylation state itself does not seem to be autonomously preserved following DNA synthesis. For group A genes, removal of methylation automatically brings about local histone acetylation, suggesting that the deacetylated histone state normally associated with these genes is actually maintained by the underlying DNA methylation pattern. Similarly, in the case of group B genes, even when DNA methylation is removed (PM cells), TSA-mediated gene activation is not stably preserved (Fig. 2, A and C). This indicates that the additional layers of repression associated with these genes must also

**FIGURE 3. Histone modification profiles.** A, PCR analysis using primers to detect promoter regions (within 1000 bp upstream of the mRNA start site) was carried out both semi-quantitatively (left panel) on Input or Bound fractions using two concentrations (1 or 3 μl) (see supplemental Fig. S2 for examples) and quantitated by phosphorimaging (“Experimental Procedures”) and by real-time PCR (right panel). Changes in histone modification of more than 2-fold between P and PM cells are indicated in red. The degree of enrichment was calculated with reference to Actb (see supplementary Fig. S2). In many cases, several primer pairs for an individual gene were used and the results averaged. The column labeled RT indicates the replication time of each gene as determined by S phase fractionation analysis (see Fig. 4). All genes replicate either in early (E), middle (M), or late (L) S phase. ND indicates that PCR reactions could not be detected with the primers tested. The gene Pgf was originally detected as belonging to group B by expression analysis but revealed a histone modification pattern more fitting to group A genes when tested by real-time PCR. B, changes in histone modification (PM/P) arranged over both assay methods are shown in graphic form for all of the genes analyzed. Genes that replicate in early S phase are marked in red, whereas late or middle replicating genes are marked in blue.
be brought about by underlying histone deacetylation-maintenance mechanisms similar to DNA methylation (Fig. 5).

Late replication timing represents a clear-cut epigenetic mark that is constitutively preserved in dividing cells (7). Although its role as a causative agent is not yet fully established, it has recently been shown to inhibit gene expression through histone deacetylation (9), making it another potential candidate for a basic repression mechanism. On the basis of this idea, we envision that multilayer repression may work in the following manner: Housekeeping genes all replicate in early S phase (7) and thus have a normal tendency to be packaged with acetylated histones following replication. The same seems to be true for group A genes, but in this case the presence of DNA methylation would recruit histone deacetylases that actively cause local deacetylation (25), thus counteracting the ground level effect of early S phase replication.

Strikingly, 50% of the group B genes in our survey replicate late in S phase (Fig. 3, A and B). Because these genes are also methylated, they carry not one but at least two fundamental repression layers. Thus, when DNA methylation is removed (Dnmt1− cells), methyl groups are no longer present to recruit deacetylase activity, but the overlying histones could still remain unacetylated by virtue of late replication timing, which automatically packages this DNA with deacetylated histones following DNA synthesis (9). The overall replication timing pattern does not appear to be dependent on DNA methylation (Ref. 29 and see legend to Fig. 4), and conversely, methylation is not necessarily influenced by the replication timing profile as can be inferred from the data in Fig. 3. Thus, these two epigenetic mechanisms are clearly set up independently.

Genes in group B that do not replicate in late S must be subject to other mechanisms of repression. One possibility is that these genes are inactivated by protein or RNA repression factors that interact with nearby regulatory elements (24). Thus, these genes would also have two independent mechanisms for being packaged in a relatively closed structure, DNA methylation (like group A) and transcriptional repressors capable of recruiting histone deacetylases. One example of this may be Oct4, which is early replicating (30) and has been shown to be inactivated by both DNA methylation (31) and protein factor-mediated repression (32). RE1-silencing transcription factor-mediated repression of nervous system-specific genes may also work in a similar manner (33).

Although our studies have concentrated almost exclusively on histone acetylation, it should be noted that this parameter merely serves as a convenient indicator of overall chromatin structure. Indeed, it is likely that all of the fundamental repression mechanisms (e.g. DNA methylation, late replication timing, and sequence-mediated repressor binding) can affect gene expression through multiple modalities, such as direct effects on transcription, histone modification, nucleosome positioning, nuclear localization, and higher order chromatin structure (24). In this context, histone deacetylation may represent only one small component of the repression machinery, and it is probably for this reason that TSA treatment only brings about partial gene activation.

**Histone Methylation**—By genetically manipulating the DNA methylation pattern of the genome, we have uncovered an interesting relationship between this epigenetic marker and the methylation of histone H3 lysine 4. All of the methylated DNA sequences examined in this study were found to be uniformly packaged with unmethylated H3(K4), and the removal of these methyl groups appears to be sufficient for bringing about rem-
ethylation of H3(K4). This dynamic correlation is consistent with the observation that unmethylated CpG island promoters are packaged with me-H3(K4) regardless of their activity state (data not shown and Ref. 27). The mechanism for how DNA methylation may influence histone methylation is not known. One possibility is that the presence of DNA methyl moieties themselves prevents local H3(K4) modification. Alternatively, DNA methyl groups could recruit enzymes capable of demethylating this site (34, 35). In any event, H3(K4) modification is clearly a direct reflection of the natural DNA methylation state in a manner that appears to be independent of both gene activity and other histone modifications.

Methylation of H3(K4) by itself is apparently insufficient to cause changes in gene expression, but it is very possible that this modification only operates in conjunction with histone acetylation. This might explain, for example, why group B genes can cause changes in gene expression, but it is very possible that this demethylation may influence histone methylation is not known. This is consistent with the idea that individual histone-marked genes poised for activation are marked by methylation. This might explain, for example, why group B genes can serve as a reflection of their underlying epigenetic mechanisms, as predicted by the histone code hypothesis (37).

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REFERENCES

1. Cedar, H. (1988) Cell 53, 3–4
2. Kafri, T., Ariel, M., Brandeis, M., Shemer, R., Urven, L., McCary, J., Cedar, H., and Razin, A. (1990) Genes Dev. 6, 705–714
3. Bird, A. P., and Wolffe, A. P. (1999) Cell 99, 451–454
4. Siegfried, Z., Eden, S., Mendelsohn, M., Feng, X., Tzubari, B., and Cedar, H. (1999) Nat. Genet. 22, 203–206
5. Razin, A. (1998) EMBO J. 17, 4905–4908
6. Struhl, K. (1999) Cell 98, 1–4
7. Goren, A., and Cedar, H. (2003) Nat Rev. Mol. Cell Biol. 4, 25–32
8. Lercher, M. J., Urrutia, A. O., and Hurst, L. D. (2002) Nat. Genet. 31, 180–183
9. Zhang, J., Feng, X., Hashimshony, T., Keshet, I., and Cedar, H. (2002) Nature 420, 198–202
10. Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T., and Weinberg, R. A. (1994) Curr. Biol. 4, 1–7
11. Li, E., Bestor, T. H., and Jaenisch, R. (1992) Cell 69, 915–926
12. Hebbes, T. R., Clayton, A. L., Thorne, A. W., and Crane-Robinson, C. (1994) EMBO J. 13, 1823–1830
13. Weber, M., Davies, J. J., Wittig, D., Oakeley, E. J., Haase, M., Lam, W. L., and Schubeler, D. (2005) Nat. Genet. 37, 853–862
14. Keshet, I., Schlesinger, Y., Farkash, S., Rand, E., Hecht, M., Segal, E., Pikarski, E., Young, R. A., Niveleau, A., Cedar, H., and Simon, I. (2006) Nat. Genet. 38, 149–153
15. Reynaud, C., Bruno, C., Boulanger, P., Grange, J., Barbesti, S., and Niveleau, A. (1992) Cancer Lett. 61, 255–262
16. Gardiner-Garden, M., and Frommer, M. (1987) J. Mol. Biol. 196, 261–282
17. 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
18. Suzuki, H., Gabrielson, E., Chen, W., Anbazhagan, R., van Engeland, M., Weijenberg, M. P., Herman, J. G., and Baylin, S. B. (2002) Nat. Genet. 31, 141–149
19. Walsh, C. P., Chaillet, J. R., and Bestor, T. H. (1998) Nat. Genet. 20, 116–117
20. Futsch, B. W., Oshiro, M. M., Wozniak, R. J., Holtan, N., Hanigan, C. L., Duan, H., and Domann, F. E. (2002) Nat. Genet. 31, 175–179
21. De Smet, C., Lurquin, C., Lethe, B., Martelange, V., and Boon, T. (1999) Mol. Cell. Biol. 19, 7327–7335
22. Fuks, F., Burgers, W. A., Brehm, A., Hughes-Davies, L., and Kouzarides, T. (2000) Nat. Genet. 24, 88–91
23. Robertson, K. D., Alt-Si-Ali, S., Yokochi, T., Wade, P. A., Jones, P. L., and Wolff, A. P. (2000) Nat. Genet. 25, 338–342
24. Lande-Diner, L., and Cedar, H. (2005) Nature Rev. Genet. 6, 648–654
25. Eden, S., Hashimshony, T., Keshet, I., Thorne, A. W., and Cedar, H. (1998) Nature 394, 842–843
26. Ekwall, K., Olsson, T., Turner, B. M., Cranston, G., and Allshire, R. C. (1997) Cell 91, 1021–1032
27. Hashimshony, T., Zhang, J., Keshet, L, Bustin, M., and Cedar, H. (2003) Nat. Genet. 34, 187–192
28. Auzuara, V., Brown, K. E., Williams, R. R., Webb, N., Dillon, N., Festenstein, R., Buckle, V., Merkenschlager, M., and Fisher, A. G. (2003) Nat. Cell Biol. 5, 668–674
29. Gribnau, J., Hochledinger, K., Hata, K., Li, E., and Jaenisch, R. (2003) Genes Dev. 17, 759–773
30. Perry, P., Sauer, S., Bilton, N., Richardson, W. D., Spivakov, M., Warnes, G., Livesey, F. J., Merkenschlager, M., Fisher, A. G., and Auzuara, V. (2004) Cell Cycle 3, 1645–1650
31. Gidekel, S., and Bergman, Y. (2002) J. Biol. Chem. 277, 34521–34530
32. Feldman, N., Gerson, A., Fang, J., Li, E., Zhang, Y., Shinkai, Y., Cedar, H., and Bergman, Y. (2006) Nat. Cell Biol. 8, 188–194
33. Luna, K. V., Burgess, R., Prefontaine, G. G., Grane, S. H., Che, Noweth, J., Schwartz, P., Pezvner, P. A., Glass, C., Mandel, G., and Rosenfeldt, M. G. (2002) Science 298, 1747–1752
34. Shi, Y., Lan, F., Matson, C., Mulligan, P., Wethstine, J. R., Cole, P. A., and Casero, R. A. (2004) Cell 119, 941–953
35. Lee, M. G., Wynder, C., Cooch, N., and Shekhattar, R. (2005) Nature 437, 432–435
36. Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S. L., and Lander, E. S. (2006) PLoS Biol. 4, 67–71
37. Jenuwein, T., and Allis, C. D. (2001) Science 293, 1074–1080
38. Gruenbaum, Y., Stein, R., Cedar, H., and Razin, A. (1981) FEBS Lett. 123, 61–71
39. Lande-Diner, L., Zhang, J., Hashimshony, T., Auzuara, V., Keshet, I., and Cedar, H. (2004) Cold Spring Harbor Symp. Quant. Biol. 69, 131–138
40. Hiratani, I., Leskovar, A., and Gilbert, D. M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 16861–16866

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